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Directory of Short Term Tests for Health and Ecological Effects

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Research Triangle Park NC 27711

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Directory of Short Term Tests for Health and Ecological Effects

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This directory provides basic information on the short term tests for health and ecological effects being performed by various U.S. EPA Laboratories through the Office of Health and Ecological Effects. The test systems are cross-indexed.

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By the

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This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. Mention of specific contractors does not necessarily indicate either exclusive engagement or recommendation.

FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

Historically, the Health Effects Research Laboratory has made a strong commitment to the development and implementation of short-term tests for potential health effects of environmental agents.

This commitment is evidenced by the formation within this laboratory of two new programs; the Genetic Toxicology Program and the Neurotoxicology Program. These programs will require redirection and commitment of new resources to develop techniques that rapidly evaluate pure chemicals and complex environmental samples for possible genotoxic and neurotic hazard.

Research on short-term tests for health and ecological effects is advancing rapidly throughout the Office of Health and Ecological Effects. This document should prove of significant value in maintaining coordination of the research program during this growth period.

F. G. Hueter, Ph.D.
Director
Health Effects Research Laboratory

PREFACE

At the request of Dr. Delbert Barth, Deputy Assistant Administrator for the Office of Health and Ecological Effects (OHEE), the first OHEE Workshop on Short-Term Tests for Health and Ecological Effects was held January 18-20, 1978, at the U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park (HERL-RTP), North Carolina.

The need for such a meeting, especially in the area of genetic toxicology, was suggested by Dr. Alexander Malcolm of the U.S. Environmental Protection Agency at Narragansett, Rhode Island. The Workshop was planned and coordinated by Dr. Frode Ulvedal and Dr. George Armstrong of OHEE with the assistance of Dr. Malcolm and the staff of the Biochemistry Branch, HERL-RTP.

The following objectives of the Workshop were established and transmitted with Dr. Barth's letter of invitation to the seven OHEE laboratories and to the National Center for Toxicological Research:

- Produce a directory listing OHEE's screening system efforts with the address and telephone numbers of the key people associated with these efforts;
- Initiate a continuing dialogue among the various investigators as well as visitation among sister laboratories;
- Formulate an agreement for the coordination of the Agency's efforts in health and ecological bioassays; and
- Identify OHEE's needs for future research in this area.

A major initiative of the Workshop was the preparation of a directory of the short-term tests currently being performed throughout OHEE. This document, the Directory of Short-Term Tests for Health and Ecological Effects, provides

PREFACE (continued)

information on the tests themselves, the laboratories where they are being performed, and the key individuals involved. It should prove to be an important Agency and interagency reference to this rapidly growing and challenging field of scientific investigation. The Directory also seeks to further the intent of the Workshop, namely to enhance communication, collaboration, understanding, and appreciation of a major component of the U.S. Environmental Protection Agency's research program.

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Chief, Biochemistry Branch
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ABBREVIATIONS

ERL-COR	Environmental Research Laboratory-Corvallis, Oregon
ERL-DUL	Environmental Research Laboratory-Duluth, Minnesota
ERL-GB	Environmental Research Laboratory-Gulf Breeze, Florida
ERL-NAR	Environmental Research Laboratory-Narragansett, Rhode Island
HERL-CIN	Health Effects Research Laboratory-Cincinnati, Ohio
HERL-RTP	Health Effects Research Laboratory-Research Triangle Park, North Carolina
NCTR	National Center for Toxicological Research, Jefferson, Arkansas
OAWM	Office of Air and Waste Management
OEMI	Office of Energy, Minerals, and Industry
OHEE	Office of Health and Ecological Effects
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
OTS	Office of Toxic Substances
OWHM	Office of Water and Hazardous Materials
U.S. EPA	United States Environmental Protection Agency

ACKNOWLEDGMENT

The cooperation of each Workshop participant and each Directory contributor are gratefully acknowledged.

Special thanks are extended to the three science editors, Dr. Shahbeg Sandhu of HERL-RTP, Dr. Jeffery Charles of HERL-RTP, and Dr. James Mckim of ERL-DUL, for their extensive efforts in screening and coordinating the contributions to the subject areas of Health Effects-Genetic Toxicology, Health Effects-General and Perinatal Toxicology, and Ecological Effects, respectively.

Our sincere appreciation is also extended to Northrop Services, Inc., for indexing, editing, and typing the Directory, and in particular to Olga Wierbicki, who coordinated the effort.

NOTE REGARDING THE TEST SYSTEMS

The test system information contained in this document was provided by the workshop participants and their colleaques. The science editors, Dr. Shahbeg Sandhu, Dr. Jeffrey Charles, and Dr. James Mckim, combined data where possible, questioned obvious errors and missing information, and insured uniformity to the extent possible in data sheets and indices. No effort was made to restrict inclusion of any test system or related information. Selection and interpretation of terms related to status of development, applications, complexity (simplest to most complex on a scale of 1 to 4), program office support, etc., were the responsibility of the submitters of the information.

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1111 INTEGRATED SYSTEM: LUNG ORGAN CULTURE SYSTEM, TISSUE HOMOGENATES, PURIFIED ENZYME SYSTEMS

Biological Activity Detected: Toxicity.

Principle: Enzyme inhibition and/or induction.

Endpoints: Qualitative: Alteration in enzyme activities and/or concentration of metabolites. Quantitative: Degree of alteration in enzyme activities and/or concentration of metabolites.

Strengths: Excellent indicator for pulmonary fibrosis; Very sensitive early indicators.

Weaknesses: Lacks in specificity in some cases; Terminal; Difficult to extrapolate to human situation.

Status of Development: Being implemented.

Describe: Test systems have been fully developed. Data are being collected.

Applications: Multimedia.

Samples: Pure Chemicals: NO_2, SO_2, Hg, Cd, Mn, Zn, Cu. Complex Mixtures: Transportation Related - diesel.

Duration: 5 years ending in 1979.

Cost/sample or chemical: $85.

Interpretation: This system is a very sensitive measure for the degree of alteration relative to pulmonary fibrosis.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Functional Pathology Branch, Biochemistry Section.

Persons to Contact: S.D. Lee, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7442).

Grant/Contract Laboratory Involved and Principal Investigators: U. of California Medical Center, San Francisco, CA 94132, R.S. Bhatnagar.

Program Office Support: OPP; OEMI.

References: 1) Bhatnagar, R.S. The Role of Superoxide in Oxidant-Induced Pulmonary Fibrosis. In: Biochemical Effects of Environmental Pollutants. S.D. Lee, ed., Ann Arbor Science Publishers, Ann Arbor, MI, 1977. 2) Hussain, M.Z., R.S. Bhatnagar, and S.D. Lee. Biochemical Mechanisms of Interaction of Environmental Metal Contaminants with Lung Connective Tissue. In: Biochemical Effects of Environmental Pollutants. Ann Arbor Science Publishers, Ann Arbor, MI, 1977.

1112 INTEGRATED SYSTEM: TISSUE HOMOGENATES, PURIFIED ENZYME SYSTEMS

Biological Activity Detected: Toxicity.

Principal: Enzyme inhibition and/or induction.

Endpoints: Qualitative: Alteration in enzyme activity and/or concentration of metabolites. Quantitative: Degree of alteration in enzyme activity and/or concentration of metabolites.

Strengths: Early indicators; Probably more sensitive than any other method.

Weaknesses: Lacks in specificity in many cases; Terminal; Difficult to extrapolate to human situation.

Status of Development: Validated.

Describe: Data are being accumulated to validate toxic effects of specific pollutants.

Applications: Multimedia.

Samples: Pure Chemicals: O_3, NO_2, Hg, Cd, SO_2. Complex Mixtures: Ambient - O_3 + SO_2.

Duration: 3 years ending June 1978.

Cost/sample or chemical: $75.

Interpretation: This system provides sensitive early indicators for metabolic/cellular injury and recovery.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Biochemistry Section.

Persons to Contact: S.D. Lee, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7442).

Grant/Contract Laboratory Involved and Principal Investigators: U. of California School of Medicine, Los Angeles, CA 90032, M.G. Mustafa.

Program Office Support: OHEE; OPP.

References: 1) Mustafa, M.G., and S.D. Lee. Pulmonary Biochemical Alterations Resulting from Ozone Exposure. Ann. Occup. Hyg., 19:17-26, 1976. 2) Mustafa, M.G., A.D. Hacker, J.J. Ospital, N. Elsayed, and S.D. Lee. Prophylactic Effect of Dietary Vitamin E on the Metabolic Response of Lung Tissue to Low-Level Ozone Exposure. Amer. Rev. Resp. Dis., 113:98, 1976. 3) Hacker, A.D., N. Elsayed, M.G. Mustafa, J.J. Ospital, and S.D. Lee. Effects of Short-Term Nitrogen Dioxide Exposure on Lung Collagen Synthesis. Amer. Rev. Resp. Dis., 113:107, 1976. 4) Ospital, J.J., N. Elsayed, A.D. Hacker, M.G. Mustafa, and D.F. Tierney. Altered Glucose Metabolism in Lungs of Rats Exposed to Nitrogen Dioxide. Amer. Rev. Resp. Dis., 113:108, 1976. 5) Lee, S.D., and M.G. Mustafa. Influence of Dietary Antioxidants in Low Level Oxidant Exposure. Presented at 4th International Clean Air Congress, Tokyo, Japan, May, 1977. 6) Mustafa, M.G., A.D. Hacker, J.J. Ospital, M.Z. Hussain, and S.D. Lee. Biochemical Effects of Environmental Oxidant Pollutants in Animal Lungs in Biochemical Effects of Environmental Pollutants. S.D. Lee, ed., Ann Arbor Science Publishers, Ann Arbor, MI, 1977. 7) Mustafa, M.G., and S.D. Lee. Biological Effects of Environmental Pollutants: Methods for Assessing Biochemical Changes. In preparation.

1113 INSTRUMENTAL METHODS OF DETECTING FUNCTIONAL AND METABOLIC DAMAGE TO TARGET TISSUES

Biological Activity Detected: Toxicity.

Principle: Increased functional activity of a tissue requires energy. Consequently, if a tissue's functional activity is stimulated, ATP is hydrolyzed to ADP and P_i which in turn stimulates oxidation of substrate and resynthesis of ATP. These metabolic changes may be observed as increases in oxygen consumption, substrate utilization or as metabolic transients induced in the electron carriers directly in tissues, in-vitro. The kinetics of these metabolic responses to stimulation are sensitive to a wide variety of chemical agents with varying mechanisms of action with both in-vitro and in-vivo treatments.

Endpoints: To this point in time the test has only been developed for brain tissue. Responses are measured in response to electrical pulses or elevation in K concentrations. Qualitative: N/A. Quantitative: Transient redox changes in NAD(P)H, fp, cyt a, b, c; Substrate utilization; Oxygen consumption; Lactic acid output; Neurotransmitter release; Amino acid metabolism; Electrical threshold; Frequency response.

Strengths: In-vitro results may be directly confirmed in-vivo with same parameters; Applicable to a wide variety of mechanisms; Applicable to all aerobic tissues; Involves measurement of the kinetics of going from a resting to an excited state rather than the steady state, thereby greatly increasing sensitivity; Applicable to very small tissue samples (2 to 3 mg).

Weaknesses: Does not lend itself to immediate identification of mechanisms unless there is a direct effect on energy metabolism proper.

Status of Development: Validated.

Describe: The test has been validated with a wide variety of inhibitors of energy and membrane active compounds such as ouabain and saxitoxin. In-vitro and in-vivo treatments with lead, methyl mercury, and alkyltin compounds indicate equivalent or more sensitive measures of effect than other parameters which have been applied to these problems.

Applications: Multimedia.

Samples: Pure Chemicals: All classes. Complex Mixtures: Industrial; Energy Related; Transportation Related.

Duration: 2 weeks/compound in-vitro.

Cost: Not precisely established, estimate $1,000 to $2,000/compound.

Interpretation: The test basically assesses potential for neurotoxicity. It indicates the effect of a chemical somewhere between functional activity and the metabolism induced by functional activity. Further studies are required to determine if the effect is on function or metabolism.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213).

1113 INSTRUMENTAL METHODS OF DETECTING FUNCTIONAL AND METABOLIC DAMAGE TO TARGET TISSUES (continued)

Grant/Contract Laboratory Involved and Principal Investigators: In-house.

Program Office Support: OHEE.

References: 1) Bull, R.J., and A.J. Trevor. J. Neurochem. 10:999-1009, 1972. 2) Bull, R.J., and A.J. Trevor. J. Neurochem., 19:1011-1022, 1972. 3) Cummins, J.T., and R.J. Bull. Biochem. Biophys. Acta, 253:29-38, 1971. 4) Bull, R.J., and J.T. Cummins. J. Neurochem., 21:923-937, 1973. 5) Bull, R.J., and S.D. Lutkenhoff. Neuropharmacol., 14:351-359, 1975. 6) Bull, R.J. J. Neurochem., 26:149-156, 1976. 7) Bull, R.J., P.M. Stanaszek, J.J. O'Neill, and S.D. Lutkenhoff. Envir. Hlth. Perspect., 12:89-95, 1975.

1114 TRACHEAL MUCOCILIARY TRANSPORT

Biological Activity Detected: Toxicity.

Principle: Particulates or gases which are deposited on the ciliated epithelium of conducting airways could adversely affect the major functions of this tissue — that of clearance of particulates and effete cells from the lung.

Endpoints: Qualitative: Cytological and histological examination of trachea exposed to air pollutants in-vivo or in-vitro. Quantitative: Measurement of ciliary beating frequency after in-vitro or in-vivo exposure.

Strengths: The in-vitro exposure model permits rapid dose-response studies for ranking of toxicity which can be followed with rapid dose-response studies after in-vivo exposure; The test is quite sensitive; The in-vivo exposure testing dose-response effects permits results to be used for standard setting and regulatory purposes; The in-vitro exposure model requires small amounts of pollutant sample.

Weaknesses: For the inhalation exposure, relatively large amounts of pollutant sample are required.

Status of Development: Validated.

Describe: Both the in-vitro and in-vivo exposure model have been successfully used for Ni, Cd, H_2SO_4, and carbon, and pollutant mixtures. The model has not been used for screening purposes.

Applications: Air.

Samples: Pure Chemicals: Any chemical likely to be deposited on conducting airways. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other - any gas, particulate or combination thereof.

Duration: In-vitro exposure: 2 weeks/dose-response of 1 chemical; In-vivo exposure: 4 weeks/dose-response of 1 chemical.

Cost: Approx. $4,000/dose-response of 1 chemical in-vivo; $2,000/dose-response of 1 chemical in-vitro.

Interpretation: Positive result predictive of damage to clearance mechanisms of lung.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: J.A. Graham, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: IIT Research Institute, 10 West 35th Street, Chicago, IL 60616, L. Schiff; Northrop Services, Inc., P. O. Box 12313, Research Triangle Park, NC 27709, B. Adkins; Ball State University, Muncie, IN 47306, D. Adalis; U. of North Carolina, Chapel Hill, NC 27514, A. Collier.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Adalis, D., D.E. Gardner, F.J. Miller, and D.L. Coffin. Toxic Effects of Cadmium on Ciliary Activity Using a Tracheal Ring Model System. Envir. Res., 13:111-120, 1977. 2) Collier, A.M., and J.B. Baseman. Organ Culture Techniques with Mycoplasma. Ann. N.Y. Acad. Sci., 225:277-289, 1973. 3) Donnelly, G.M., H.F.

1114 TRACHEAL MUCOCILIARY TRANSPORT (continued)

McKean, C.S. Heird, and J. Green. Ciliostasis as a Bioassay. Arch. Envir. Hlth., 28:350-355, 1974.

1115 PLATELET SECRETION MEASURED BY ATP RELEASE

Biological Activity Detected: Toxicity; Pharmacologic modulation.

Principle: Platelet function is important in thrombosis, shock, and most inflammatory reactions. Platelet secretion accompanies the more commonly measured aggregation response, and secretion is more easily measured than aggregation.

Endpoints: Qualitative: N/A. Quantitative: ATP release from platelet suspensions is measured by Lucifirin-luciferase assay.

Strengths: Fast; Reproducible; Does not require expensive equipment; Applicable to studies of human blood; Can employ both in-vivo and in-vitro exposures.

Weaknesses: Have not yet been determined.

Status of Development: Developmental.

Describe: Procedures have not been fully developed.

Applications: Air; Water; Food.

Samples: Pure Chemicals: Most classes. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related.

Duration: 1 month to develop in-vitro technique; 3 months to develop in-vivo technique; approx. 1 week to implement.

Cost: For development $15,000; to test chemical less than $5,000/chemical.

Interpretation: A positive result is predictive of possible platelet dysfunction.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: G. Hatch, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Charo, O.F., R.D. Feinman, and T.C. Detwiler. J. Clin. Invest., 60:866-873, 1977.

1116 MAMMALIAN PLATELET AND FIBROBLAST IMPAIRMENT

Biological Activity Detected: Toxicity.

Principle: Contaminant is added to platelets in-vitro. The relative amounts of energy metabolism intermediates are measured. C^{14} adenine is used as a precursor.

Endpoints: Qualitative: Ratios of ATP, ADP, and AMP. Quantitative: N/A.

Strengths: Quantitative; Rapid; Capable of direct interpretation.

Weaknesses: Sensitivity.

Status of Development: Developmental.

Describe: Testing with dilutions of pure compounds and extracts of wastewater.

Applications: Water.

Samples: Pure Chemicals: Hydrocarbons. Complex Mixtures: Ambient - rivers; Other - wastewaters.

Duration: 3 years.

Cost/sample or chemical: $125.

Interpretation: Positive result suggests a possible interference of platelet function in-vivo.

Level of Complexity: I.

OHEE Laboratory Involved: HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: H. Pahren, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7217).

Grant/Contract Laboratory Involved and Principal Investigators: U. of Colorado Medical Center, 4200 E. 9th Ave., Denver, CO 80262, C.C. Solomons.

Program Office Support: OHEE.

References: Not yet available.

1117 LYMPHOCYTE CYTOTOXICITY

Biological Activity Detected: Toxicity.

Principle: Lymphocytes (T cells) have been shown to have cytotoxic effector actions against neoplastic or other cells. The potential exists that pollutants could adversely affect this function, thereby increasing the risk of the host to the development of neoplastic disease.

Endpoints: Qualitative: N/A. Quantitative: Measurements of lymphocyte cytotoxic activity and lectin induced transformation will be made following in-vitro pollutant exposure.

Strengths: The in-vitro model would permit rapid screening for a significant health parameter; Dose-response studies would permit ranking of pollutant effects; Relatively small quantities of pollutant would be required.

Weaknesses: The in-vitro model is not yet validated. Even after validation, results would have to be confirmed with in-vivo exposure studies, possibly of a chronic nature, before the data could be useful for regulation.

Status of Development: Developmental.

Describe: Highly developmental. No pollutants have yet been tested. Model to be completed March, 1979.

Applications: Multimedia.

Samples: Pure Chemicals: Potentially, any chemical. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other - any mixture that does not include gas.

Duration: Not yet determined.

Cost: $175,000/model development.

Interpretation: In-vitro model not validated at present.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: J.A. Graham, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: Rockefeller U., 1230 York Ave., New York, NY 10021, M. Bowers.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Kirchner, H., and R.M. Blaese. In Lymphocyte Recognition and Effector Mechanisms. Acad. Press, N.Y., 1974. pp. 357-361. 2) Lightbody, J., and J.C. Rosenberg. In Lymphocyte Recognition and Effector Mechanisms. Acad. Press, N.Y., 1974. pp. 363-367. 3) Perlman, P., and G. Holm. Adv. Immunol., 11: 117, 1969.

1118 IMPAIRMENT OF NEUTROPHIL PHAGOCYTOSIS

Biological Activity Detected: Toxicity.
Principle: Functional and metabolic disturbance of neutrophils are measured after exposure to contaminant.
Endpoints: Qualitative: Percent phagocytosis and percent killing power are measured. Quantitative: N/A.
Strengths: Rapid; Dose-response of procedure has been shown.
Weaknesses: Sensitivity may be a possible problem if low PPB exposure is necessary.
Status of Development: Developmental.
Describe: Testing with dilutions of pure compounds and extracts of wastewater.
Applications: Water.
Samples: Pure Chemicals: Hydrocarbons. Complex Mixtures: Ambient - rivers; Other - wastewaters.
Duration: 3 years.
Cost/sample or chemical: Not yet determined.
Interpretation: Positive result is predictive of possible damage to neutrophils in-vivo.
Level of Complexity: 3.
OHEE Laboratory Involved: HERL-CIN, Field Studies Division, Toxicological Assessment Branch.
Persons to Contact: H. Pahren, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7217).
Grant/Contract Laboratory Involved and Principal Investigators: U. of Colorado Medical Center, 4200 E. 9th Ave., Denver, CO 80262, W.L. Weston.
Program Office Support: OHEE.
References: 1) Tan, J.S., et al. A Modified Assay of Neutrophil Function: Use of Lysostrophin to Differentiate Defective Phagocytosis from Impaired Intracellular Killing. J. Lab. Clin. Med., 78:316, 1971.

1119 HUMAN LUNG FIBROBLASTS (WI38)

Biological Activity Detected: Toxicity.

Principle: Toxicants alter biosynthetic processes leading to a reduction in cell growth and division.

Endpoints: Qualitative: Morphology. Quantitative: Cell number and viability; Total cell protein and DNA; Cell adenosine triphosphate; Incorporation of radio-labelled thymidine, uridine, and leucine.

Strengths: Relatively inexpensive; Rapid; Fewer samples required than for conventional whole animal bioassays; One of the best characterized diploid human cells available for toxicity bioassays.

Weaknesses: Not representative of intact animals, providing only preliminary information about the potential health hazards of the test chemicals; May be replaced by other cell strains as supplies dwindle; The system currently can not be coupled with mutagenecity testing unlike other mammalian cell systems.

Status of Development: Being implemented.

Describe: A number of pure compounds have been evaluated.

Applications: Air; Water.

Samples: Pure Chemicals: Inorganics, Organics, Heavy Metals.
Complex Mixtures: Industrial; Energy Related - fly ash; Other - AWT effluent, metal-coated fly ash.

Duration: 20 hr.

Cost/sample or chemical: $500 to $1,000.

Interpretation: Alterations in the basic metabolic processes and cellular structure indicate the potential toxicity of the agent.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Field Studies Division, Toxicological Assessment Branch; HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.

Persons to Contact: N.E. Kowal, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7477); M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); J.L. Huisingh, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2537).

Grant/Contract Laboratory Involved and Principal Investigators: Gulf South Research Institute, P. O. Box 26518, New Orleans, LA 70186, N. Gruener; Northrop Services, Inc. P.O. Box 12313, Research Triangle Park, NC 27709, N.E. Garrett.

Program Office Support: OHEE; OEMI.

References: 1) Campbell, J.A., H.F. Stack, M.R. Williams, D. Tillery, N. Custer, B.F. Russell, S.W. King, E.B. Siegel, and N.E. Garrett. Cellular Toxicity of Four Liquid Effluent Samples from Textile Mills: Studies on the Rabbit Alveolar Macrophage, WI38 Human Fibroblast and Chinese Hamster Ovary Cell In-Vitro. Contract Report ESG-TR-78-04 to the U.S. Environmental Protection Agency, Northrop Services, Inc., Research Triangle Park, NC. February 1978. 2) Garrett, N.E., J.A. Campbell, J.L. Huisingh, and M.D. Waters. The Use of Short-Term Bioassay Systems in the Evaluation of Environmental Particulates. In: Proceedings of the Symposium on the Transfer and Utilization of Particulate Control Technology. Denver, CO, July 24, 1978. In press. 3) Waters, M.D., T.O. Vaughan, D.J. Abernathy, H.R. Garland, C.C. Cox, and D.L. Coffin. Toxicity of Platinum (IV) Salts on Cells of Pulmonary Origin. Envir. Hlth. Perspect., 12:45-56, 1975. 4) Waters, M.D., D.R. Abernathy, H.R. Garland, and D.L. Coffin. Toxic Effects of Selected Metallic Salts on Strain W138 Human Lung Fibroblasts. In-Vitro, 10:342, 1974. 5) Waters, M.D., J.L. Huisingh, and N.E. Garrett. The Cellular Toxicity of Complex Environmental Mixtures. In: Proceedings of the Symposium on the Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures. Williamsburg, VA, 1978.

11110 CHINESE HAMSTER OVARY (CHO) CLONAL TOXICITY

Biological Activity Detected: Toxicity.
Principle: The toxicity of the samples is evaluated from clonal growth.
Endpoints: Qualitative: N/A. Quantitative: Colony formation.
Strengths: The CHO cell system is well characterized and able to form discrete colonies from single cells; The cell is phagocytically active in culture and sensitive in toxic particulate materials.
Weaknesses: The cell type may not be representative of metabolically active target cells which receive exposure to environmental toxicants.
Status of Development: Being implemented.
Describe: Protocols are established and environmental samples are being evaluated.
Applications: Air; Water.
Samples: Pure Chemicals: H_2SiO_3, Ni_3S_2. Complex Mixtures: Industrial - textile mill effluents; Energy Related - fly ash; Other - particulate materials: silica, fly ash, Dolomite.
Duration: 6 days.
Cost/sample or chemical: $500 to $1,000.
Interpretation: Biochemical changes in CHO cells exposed to the particulates indicate toxicity.
Level of Complexity: 2.
OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.
Persons to Contact: J.L. Huisingh, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2537).
Grant/Contract Laboratory Involved and Principal Investigators: Northrop Services, Inc., P.O. Box 12313, Research Triangle Park, NC 27709, N.E. Garrett.
Program Office Support: OEMI.
References: 1) Hsie, A.W., et al. Quantitative Mammalian Cell Genetic Toxicology: Study of the Cytotoxicity and Mutagenicity of Seventy Individual Environmental Agents Related to Energy Technologies and Three Subfractions of a Crude Synthetic Oil in the CHO/HGPRT System. In: Proceedings of the Symposium on Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures. Williamsburg, VA, 1978. 2) Campbell, J.A., H.F. Stack, M.R. Williams, D. Tillery, N. Custer, B.F. Russell, S.W. King, E.B. Siegel, and N.E. Garrett. Cellular Toxicity of Four Liquid Effluent Samples from Textile Mills: Studies on the Rabbit Alveolar Macrophage, WI38 Human Fibroblast and Chinese Hamster Ovary Cell In-Vitro. Contract Report ESG-TR-78-04 to the U.S. Environmental Progection Agency, Northrop Services, Inc., Research Triangle Park, NC. February, 1978. 3) Garrett, N.E., J.A. Campbell, J.L. Huisingh, and M.D. Waters. The Use of Short-Term Bioassay Systems in the Evaluation of Environmental Particulates. In: Proceedings of the Symposium on the Transfer and Utilization of Particulate Control Technology. Denver, CO, July 24, 1978. In press.

11110 CHINESE HAMSTER OVARY (CHO) CLONAL TOXICITY (continued)

4) Waters, M.D., J.L. Huisingh, and N.E. Garrett. The Cellular Toxicity of Complex Environmental Mixtures. In: Proceedings of the Symposium on the Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures. Williamsburg, VA, 1978.

11111 RABBIT ALVEOLAR MACROPHAGE (RAM)

Biological Activity Detected: Toxicity.

Principle: Toxic agents alter basic metabolic processes and cellular structure of the macrophage.

Endpoints: Qualitative: Morphology. Quantitative: Cell number and viability; Cell adenosine triphosphate; Phagocytic activity; Total cell protein; Hydrolytic enzyme specific activities.

Strengths: The alveolar macrophage plays an important role in the defense of the lung against inhaled particulate materials; This cell type receives direct exposure to environmental toxicants.

Weaknesses: This in-vitro cell system approximates the response which might be observed in the intact animal.

Status of Development: Being implemented.

Describe: The response of the RAM system to a variety of industrial and energy-related particulates has been studied.

Applications: Air; Water.

Samples: Pure Chemicals: Metal chlorides and sulfates, Metallic oxides. Complex Mixtures: Industrial - textile effluents, aluminum refinery, copper smelter; Energy Related - coal gasification and fluidized bed combustion; Other - metal-coated fly ash.

Duration: 20 hr.

Cost/sample or chemical: $500 to $900.

Interpretation: Changes in cellular adenosine triphosphate and viability by dye exclusion indicate potential toxicity of tested substance.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.

Persons to Contact: M.D. Waters, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); J.L. Huisingh, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2537).

Grant/Contract Laboratory Involved and Principal Investigators: IIT Research Institute, 10 West 35th Street, Chicago IL 60616, C. Aranyi: Northrop Services, Inc., P.O. Box 12313, Research Triangle Park, NC 27709, N.E. Garrett.

Program Office Support: OHEE; OEMI; OAWM.

References: 1) Huisingh, J.L., J.A. Campbell, and M.D. Waters. Evaluation of Trace Element Interactions Using Cultured Alveolar Macrophages. In: Pulmonary Macrophage and Epithelial Cells. Conf-760972, Sanders, C.L., R.P. Schneider, G.E. Dagle, and H.A. Hagan, eds. ERDA Symposium Series 43, Technical Information Center, Energy Research and Development Administration, 1977. pp. 346-357. 2) Waters, M.D., J.L. Huisingh, and N.E. Garrett. The Cellular Toxicity of Complex Environmental Mixtures. In: Proceedings of the Symposium on the Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures. Williamsburg, VA, 1978. 3) Waters, M.D., D.E. Gardner, and D.L. Coffin. Cytotoxic Effects of Vanadium on Rabbit Alveolar Macrophage In-Vitro. Toxicol. Appl. Pharmacol., 28:253-263, 1974. 4) Waters, M.D., T.O. Vaughan, J.A. Campbell, F.J. Miller, and D.L. Coffin. Screening Studies on Metallic Salts Using the Rabbit

11111 RABBIT ALVEOLAR MACROPHAGE (RAM) (continued)

phage In-Vitro. Toxicol. Appl. Pharmacol., 28:253-263, 1974. 4) Waters, M.D., T.O. Vaughan, J.A. Campbell, F.J. Miller, and D.L. Coffin. Screening Studies on Metallic Salts Using the Rabbit Alveolar Macrophage. In-Vitro, 10:342-343, 1974. 5) Waters, M.D., D.E. Gardner, C. Arnyi, and D.L. Coffin. Metal Toxicity for Rabbit Alveolar Macrophages In-Vitro. Envir. Res., 9:32-47, 1975. 6) Waters, M.D., T.O. Vaughan, D.J. Abernathy, H.R. Garland, C.C. Cox, and D.L. Coffin. Toxicity of Platinum (IV) Salts for Cells of Pulmonary Origin. Envir. Hlth. Perspect., 12:45-56, 1975.

11112 RAT HEPATOCYTE (LIVER CELL)

Biological Activity Detected: Toxicity.

Principle: Toxic agents alter basic metabolic processes and cellular structure and function of the hepatocyte.

Endpoints: Qualitative: Morphology. Quantitative: Cellular viability; Adenosine triphosphate content; Tyrosine aminotransferase activity; Total cell protein.

Strengths: These primary liver parenchyonal cells resemble the adult liver cell in-vivo morphologically and in many of the biochemical parameters evaluated.

Weaknesses: These cells do not divide and must be isolated from a rat prior to each assay. Since there is rat-to-rat variation, cells from several rats should be used to evaluate each chemical.

Status of Development: Developmental; Being implemented.

Describe: New endpoints are being developed; however, the assay is now being implemented with both inorganic and organic chemicals.

Applications: Multimedia.

Samples: Pure Chemicals: Inorganic salts, Organic solvents, Organic solids. Complex Mixtures: N/A.

Duration: 20 hr.

Cost/sample or chemical: $500 to $1,000.

Interpretation: Alterations in the basic metabolic processes and cellular structure and function of the liver cells determine the potential toxicity of the agent.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section.

Persons to Contact: J.L. Huisingh, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2537).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OTS.

References: 1) Huisingh, J.L., J.P. Inmon, L.C. King, K. Williams, and M.D. Waters. The Use of Rat Liver Parenchymal Cells in Evaluating Cellular Response to Toxic Metals and Carcinogenic Polycyclic Aromatic Hydrocarbons. In-Vitro, 13:182, 1977.
2) Waters, M.D., and J.L. Huisingh. In-Vitro Testing for Chemical Toxicity: Mammalian Target Cells. In-Vitro, 13:192, 1977.

11113 CHINESE HAMSTER OVARY (CHO) CYTOTOXICITY AND MUTAGENICITY

Biological Activity Detected: Toxicity; Mutagenicity.
Principle: Toxicity is evaluated from effects on clonal growth. Mutation at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus is determined from mutants isolated in 6-thioguanine containing media.
Endpoints: Qualitative: N/A. Quantitative: Colony formation (cytotoxicity); Mutation frequency.
Strengths: Cytotoxicity and mutagenicity may be studied simultaneously.
Weaknesses: The cell type may not be representative of metabolically active cells which receive exposure to environmental toxicants.
Status of Development: Developmental.
Describe: This assay has been shown to be useful in studies of 70 individual environmental agents related to energy technologies and 3 subfractions of a crude synthetic oil.
Applications: Air; Water.
Samples: Pure Chemicals: Polycyclic hydrocarbons, Metallic compounds, Nitrosamines, Quinoline compounds, Physical agents, Alkylating agents, Nitrogen mustards, and Aromatic amines. Complex Mixtures: Energy Related - synthetic fuel.
Duration: 18 days.
Cost/sample or chemical: $500 to $1,000.
Interpretation: Decreasing clonal growth after exposure of CHO cells indicates potential toxicity of test substance. Increasing numbers of mutants with increasing concentration of the test substance indicate the substance is a potential mutagen.
Level of Complexity: 2
OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section.
Persons to Contact: J.L. Huisingh, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2537).
Grant/Contract Laboratory Involved and Principal Investigators: Northrop Services, Inc., P.O. Box 12313, Research Triangle Park, NC 27709, N.E. Garrett.
Program Office Support: OHEE; OEMI.
References: 1) Hsie, A.W., et al. Quantitative Mammalian Cell Genetic Toxicology: Study of the Cytotoxicity and Mutagenicity of Seventy Individual Environmental Agents Related to Energy Technologies and Three Subfractions of a Crude Synthetic Oil in the CHO/HGPRT System. In: Proceedings of the Symposium on Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures. Williamsburg, VA, 1978. 2) O'Neill, J.P., P.A. Brimer, R. Machanoff, G.P. Hirsch, and A.W. Hsie. A Quantitative Assay of Mutation Induction at the Hypoxanthine-Quanine Phosphoribosyl Transferase Locus in Chinese Hamster Ovary Cells: Development and Definition of the System. Mutat. Res., 45:91-101, 1977. 3) O'Neill, J.P., D.B. Couch, R. Machanoff, J.R. San Sebastian, P.A. Brimer, and A.W. Hsie. A Quantitative Assay of Mutation Induction at the Hypoxanthine-Guanine Phosphoribosyl Transferase Locus in Chinese Hamster Ovary Cells (CHO/HGPRT system): Utilization with a Variety of Mutagenic Agents. Mutat. Res., 45:103-109, 1977.

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1121 LD50

Biological Activity Detected: Toxicity.

Principle: Based on preliminary range finding tests, 10 dose-levels of the test compound are selected in the range from 0% to 100% mortality. Each group would consist of 7 animals. The animals receive one dose and then are observed for a period of 14 days. After the 14-day period, a dose-response (cumulative mortality) curve is plotted and the dose level producing 50% mortality is interpolated.

Endpoints: Qualitative: Clinical signs of toxicity. Quantitative: Cumulative mortality.

Strengths: Can determine a dose-response curve for nearly all test compounds and from that establish a maximum tolerated dose level to use in a multiple dose, longer-ranged study.

Weaknesses: Toxicity of vehicle; Solubility; Vehicle-compound synergism and antagonism.

Status of Development: Validated.

Describe: The chemical is administered by route of interest, condition of animals standardized, and observation period specified. Decisions, however, must be made concerning the type of solvent or vehicle that is most appropriate.

Applications: Multimedia.

Samples: Pure Chemicals: Most classes. Complex Mixtures: Drinking water concentrates.

Duration: Variable.

Cost/sample or chemical: $500.

Interpretation: The establishment of a maximum tolerated dose.

Level of Complexity: I.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Loomis, T.A. Essentials of Toxicology, 2nd Ed. Lea and Febiger, Philadelphia, PA, 1974. pp. 17-25.

1122 WHOLE ANIMAL LD50 — ORAL, DERMAL

Biological Activity Detected: Toxicity.

Principle: Administration of test substance to a sufficient number of rats, over a dosage range resulting in 0% to 100% effects.

Endpoints: Qualitative: Clinical effects. Quantitative: Effective dosage to produce effect, LD50 value.

Strengths: Estimates relative toxicity; Economical; Simple to conduct.

Weaknesses: May not apply to all species; May not correspond with data from other laboratories.

Status of Development: Being implemented.

Describe: Tests currently being conducted on limited basis on relevant selected compounds.

Applications: Multimedia.

Samples: Pure Chemicals: All classes. Complex Mixtures: Other - technical grade materials, formulated products.

Duration: 3 months.

Cost: $5,000/chemical for full battery of tests.

Interpretation: A rating of the toxicity of the various chemicals is obtained.

Level of Complexity: 1.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division.

Persons to Contact: R. Linder, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2701).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OPP.

References: 1) Gainer, T.B. Acute Toxicity of Pesticides. Toxicol. Appl. Pharmacol., 1960.

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1131 INTEGRATED SYSTEM: GENERAL CLINICAL PATHOLOGY

Biological Activity Detected: Systemic effects.

Principle: Measurement of serum constituents, proteins, enzyme activities, hematological parameters such as cell counts, cell morphology in peripheral blood and in bone marrow, measurement of special endocrinologic parameters such as thyroid and adrenal functions, pituitary hormones, etc. Diagnosis of malignancies by measurement of tumor marker proteins, serum isoenzyme patterns, etc. Measurement of urinary constituents.

Endpoints: Qualitative: Technique dependent. Quantitative: Technique dependent.

Strengths: High degree of quality control; Great amount of information is available as to the diagnostic implications of abnormal findings.

Weaknesses: Often not capable of signaling asymptomatic preclinical toxic effects; Selection of tests must be done with care and proper planning to ensure maximal effectivity.

Status of Development: Validated.

Describe: This entry encompasses approximately 800 different varieties of tests that may be performed on animals in-vivo or on biological specimens in-vitro. Usually, a battery of tests will be performed examining various organ functions.

Applications: Multimedia.

Samples: Pure Chemicals: N/A. Complex Mixtures:

Other - biological specimens: blood, urine, bone marrow.

Duration: Continuous.

Cost/sample or chemical: Test dependent.

Interpretation: The totality of a battery is aimed at detecting organ-specific toxic effects.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch, Systemic and Genetic Effects Group; HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213); J.P. Bercz, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7480).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: Theories and practical techniques as compiled in clinical pathology textbooks: 1) Henry and Davidson. Clinical Diagnosis by Laboratory Methods. 2) Tietz. Clinical Chemistry. 3) Wintrobe. Clinical Hematology.

1132 SLEEP-TIME STUDY

Biological Activity Detected: Identifies the biological activity of a compound indicating a potential for interaction with other compounds.

Principle: Chemicals that induce or inhibit MFO will alter the pharmacological effects of drugs metabolized by MFO. Hexabarbital and zoxogolamin are depressant drugs whose properties are well known in this respect. Animals are given a single dose or multiple doses of the test compound at a tolerated but effective level. Two hours after the final dose they are challenged with an anesthetic dose of hexobarbital. A control group receiving no test compound also receives the hexobarbital. The time is measured from the instant the animals lose their "righting reflex" (ability to right themselves when laid flat on their back) to the time they regain it.

Endpoints: Qualitative: Induction or inhibition of liver enzyme activity. Quantitative: Measured sleep-time.

Strengths: Fast and presumptive assay to determine whether a compound will induce or inhibit liver enzymes; Useful in planning more extensive metabolism studies.

Weaknesses: Changes in rates of metabolism must be documented to conclude the effect mediated via MFO.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: All classes. Complex Mixtures: Industrial; Energy Related.

Duration: Variable depending on projected properties of test compounds.

Cost: Approx. $200/compound.

Interpretation: The results determine the ability of a compound to induce or inhibit enzymatic systems.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch; HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213); D.E. Gardner, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Conney, A.H., et al. Adaptive Increases in Drug Metabolizing Enzymes Induced by Phenobarbital and Other Drugs. J. Pharmacol. Exp. Ther., 130:1-8, 1960. 2) LaDu, B.N., H.G. Mandel, and E.L. Way. Fundamentals of Drug Metabolism and Drug Disposition. The Williams and Wilkins Co., Baltimore, MD, 1971.

1133 METABOLISM OF CHLORINATED HYDROCARBONS IN SUBHUMAN PRIMATES

Biological Activity Detected: Comparative metabolism.
Principle: In drug metabolism studies, the primate as a model more often represents humans than any other animal model. It is likely that this is the case for environmental contaminants also.
Endpoints: Qualitative: Comparison of metabolites from various animal species. Quantitative: Quantitative analysis of metabolites from various animal species.
Strengths: The primate is most likely to be representative of man in its metabolic activity toward environmental contaminants.
Weaknesses: Expense and difficulty of working with monkeys.
Status of Development: Validated.
Describe: After dosage, specific enzymes are tested for activity. Excreta samples are chemically analyzed for metabolites.
Applications: Multimedia.
Samples: Pure Chemicals: Chlorinated aliphatic hydrocarbons, Chlorinated aromatic hydrocarbons. Complex mixtures: N/A.
Duration: 3 months.
Cost: $15,000 to $18,000.
Interpretation: Comparison of metabolism from various animal systems.
Level of Complexity: 3.
OHEE Laboratory Involved: HERL-CIN, Exposure Evaluation Branch, Organics Metabolism Section.
Persons to Contact: R.D. Lingg, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7463).
Grant/Contract Laboratory Involved and Principal Investigators: U. of Cincinnati, Cincinnati, OH 45221, C. Smith, (Commercial 513 872-5700).
Program Office Support: OHEE.
References: 1) Smith, C., R.D. Lingg, and R.G. Tardiff. Comparative Metabolism of Haloethers. Ann. N.Y. Aca. Sci., 298:111, 1977.

Biological Activity Detected: Toxicity; Biotransformation; Comparative metabolism.

Principle: Identification and quantitative analysis of major metabolites are obtained through the use of modern analytical techniques. From these results, a metabolic pathway for a selected compound can be proposed. The potential of a compound for interaction with macromolecules is better understood.

Endpoints: Qualitative: Identification of major metabolites.
Quantitative: Quantitative analysis of major metabolites.

Strengths: Leads to an understanding of the structural differences in compounds which affect their metabolic disposition and toxicity.

Weaknesses: Difficult to extrapolate to the human condition.

Status of Development: Being implemented.
Describe: Most separation and derivatization techniques have been validated using β-Chloroethers and Trichlorobenzenes as model compounds. Additional work needs to be done on processing mass spectral data.

Applications: Multimedia.

Samples: Pure Chemicals: Halogenated aliphatic hydrocarbons, Halogenated aromatic hydrocarbons. Complex Mixtures: N/A

Duration: 3 months.

Cost/sample or chemical: $10,000 to $15,000.

Interpretation: The determination of metabolic pathways leads to an understanding of the differences in the toxication of a selected chemical in various animal species.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Exposure Evaluation Branch, Organics Metabolism Section; HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Metabolic Effects Section.

Persons to Contact: R.D. Lingg, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7463); S. Nesnow, U.S. EPA, HTRL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) R.D. Lingg, et al. Fate of Bis (2-Chloroethyl) Ether in Rats after Acute Oral Administration. To be presented at the Seventeenth Annual Meeting of the Society of Toxicology, Mar. 12-16, 1978, San Francisco, CA. 2) R.D. Lingg, W. Kaylor, S.M. Pyle, and R.G. Tardiff. Thiodiglycolic Acid: A Major Metabolite of Bis (2-Chloroethyl) Ether. Submitted to Toxicol. and Appl. Pharmacol. Dec., 1977.

1135 MODEL SUBSTRATE METABOLISM

Biological Activity Detected: Toxicity.

Principle: The model substrate assay attempts to determine the effect of environmental chemicals on metabolic pathways in the living animal. This test involves repeated exposure of the experimental animals (6/treatment group) to the toxicants under investigation. After this pretreatment period the animals receive a single oral dose of the model substrate, ^{14}C-lindane. A useful variation in this assay involves the simultaneous administration of lindane with the toxicants being studied.

Endpoints: Qualitative: Altered metabolite profiles serve as a "fingerprint of toxicant exposure" since xenobiotics have characteristic effects on the metabolic pathways of the model substrate.
Quantitative: Induced or inhibited metabolism are measured by GLC analysis and liquid scintillation counting of excreted products.

Strengths: Analysis of excreted metabolites provide direct measure of overall metabolism in living animal; Changes in enzyme activity due to disruption of phospholipid membranes, loss of permeability barriers, and loss of local charge effect are avoided; Anomalous enzyme alterations caused by accumulation of substrate and/or metabolites are avoided since normal clearance mechanisms are functional; Economical and practical since comparative activity of multiple pathways are determined on individual animals; Daily changes in the metabolic pathways of animals repeatedly exposed to toxicants can be determined.

Weaknesses: Requires GLC standards of the model substrate metabolites being determined.

Status of Development: Being implemented.
Describe: The model substrate assay has been successfully employed to study interactions between a variety of environmental chemicals, nutritional stress, and the enzyme systems that detoxify or enhance the toxicity of xenobiotics. The correlation between exposure to xenobiotics, whose metabolites produce the same lesion, and their induction of a model substrate metabolite profile with a common element is being investigated.

Applications: Multimedia.

Samples: Pure Chemicals: Ca, Organochlorine pesticides, Herbicides, Insecticides, Fungosides, Drugs, Toxic substances.
Complex Mixtures: Not yet tested.

Duration: Test: 2-week exposure; Analysis: 3 weeks.

Cost: $2,700.

Interpretation: It is thought that the generation of unusual alterations in the relative activity of various metabolic pathways of a model substrate can signal toxic interactions.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.

Persons to Contact: R.W. Chadwick, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2750); M.F. Copeland, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2678).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OPP; OTS.

References: 1) Chadwick, R.W., C.J. Chadwick, J.J. Freal, and C.C. Bryden. Comparative Enzyme Induction and Lindane Metabolism in Rats Pre-treated with Various Organochlorine Pesticides. Xenobiotica, 7:235-246, 1977. 2) Chadwick, R.W., W.S. Simmons, C.C. Bryden, L.T. Chuang, L.M. Key, and C.J. Chadwick. Effect of Dietary Liquid and Dimethyl Sulfoxide on Lindane Metabolism. Toxicol. and Appl. Pharmacol., 39:391-410, 1977. 3) Chadwick, R.W., M.F. Copeland, and C.J. Chadwick. Enhanced Pesticide Metabolism, a Previously Unreported Effect of Dietary Fiber in Mammals. Food and Cosmetics Toxicol., 1978. In press.

1136 XENOBIOTIC MECHANISMS

Biological Activity Detected: Capacity of chemical to act as a synergist or antagonist.

Principle: Groups of 10 animals/sex/dose level are exposed to test compounds for periods of 3 to 7 days, to test the potential of the compound to induce or inhibit liver enzymes. Dose levels used are the maximum tolerated dose (MTD), 1/2 MTD and 1/4 MTD. Following the dose regimen the animals are sacrificed and liver samples are taken for the enzyme assays.

Endpoints: Qualitative: Hexabarital cytP-450 levels, cytPc reductase, O-demethylase, and other microsomal enzyme activities. Quantitative: Hexabarital cytP-450 levels, cytPc reductase, O-demethylase, and other microsomal enzyme activities.

Strengths: Quick assay to determine drugs' potential to cause liver damage; Does not require large numbers of animals.

Weaknesses: No significant weaknesses.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: Xenobiotics. Complex Mixtures: N/A

Duration: Variable, 1 week to chronic, depending on whether testing is acute or chronic.

Cost/sample or chemical: Variable, depending on test. 1 week at $35,000/manyear equals approximately $700.

Interpretation: This test determines the potential for synergisms and/or antagonisms mediated through altered xenobiotic metabolisms.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch; HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Metabolic Effects Section.

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213); S. Nesnow, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Hayakwa, T. A Simple Radioisotope Assay for Microsomal Aryl Hydroxylase. Anal. Biochem., 51:501-509, 1973. 2) Dallner, G. Studies on the Structural and Enzymatic Organization of Liver Microsomes. Acta Path. Scand., 166:7-41, 1963. 3) Neal, R.A. Studies on the Mechanisms of Detoxification of Cholinergic Phosphorothioates. J. Pharmacol. Exp. Therap., 148:185-192, 1956. 4) Lucier, G.W. Microsomal Rat Liver UDP Glucuronyl Transferase: Effects of Piperonyl Butoxide. Arch. Biochem. and Biophys., 145: 520-530, 1971. 5) Omura, T. The Carbon Monoxide Binding Pigment of Liver Microsomes: I. Evidence for its Hemoprotein Nature. J. Biol. Chem., 239;2370-2378, 1964.

1137 OXIDANT PRODUCTION BY LEUKOCYTES AND ALVEOLAR MACROPHAGES MEASURED BY CHEMILUMINESCENCE

Biological Activity Detected: Toxicity.

Principle: Oxidant production in alveolar macrophage microbicidal activity is due to alveolar macrophage metabolic activation. Oxidants are measured by measuring light produced by oxidation reactions.

Endpoints: Qualitative: N/A. Quantitative: Production of light as measured in an ATP photometer. The amount of light can be measured in response to purified macrophage stimulates and particles.

Strengths: Very reproducible; Fast; Simple; Does not kill cells; Requires very few cells; Applicable to human blood cells; Several types of oxidant can be measured; In-vivo or in-vitro dose-response tests can be made; In-vivo tests are suitable for standard-setting and regulatory purposes.

Weaknesses: Chemiluminescent reactions are susceptible to many quenching effects and competing reactions; Adequate controls are necessary; For in-vivo exposures, relatively large amounts of pollutant sample are required.

Status of Development: Being implemented.

Describe: Effects of O_3, NO_2, and various particles are being determined.

Applications: Multimedia.

Samples: Pure Chemicals: All transparent chemicals which are not readily oxidized. Complex Mixtures: All materials except those which are very opaque.

Duration: In-vitro exposure: 2 weeks/dose-response of 1 chemical; in-vivo exposure: 4 weeks/dose-response of 1 chemical.

Cost: In-vitro exposure: $5,000/chemical; In-vivo exposure: $10,000/chemical.

Interpretation: Defect in oxidant production predicts possible microbicidal defect in the macrophages.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: G. Hatch, U.S. EPA, HERL-RTP, Biomedical Research Branch, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Hatch, G.E., D.E. Gardner, and D.B. Menzel. J. Exp. Med., 147;183-195, 1978. 2) Rosen, H., and S.J. Klebanoff. J. Clin. Invest., 58:50, 1976.

1138 CYCLIC NUCLEOTIDE CONCENTRATIONS IN LEUKOCYTES AND ALVEOLAR MACROPHAGES

Biological Activity Detected: Toxicity; Presumptive oncogenicity; Pharmacologic modulation of promotion.

Principle: Cyclic AMP and Cyclic GMP are intracellular hormones which modulate cellular functions, including cell proliferation, secretion, and movement. The hormones are measured by radioimmunoassay.

Endpoints: Qualitative: N/A. Quantitative: Concentrations of cyclic nucleotide per cell correlate with cellular activity.

Strengths: Cyclic nucleotide concentrations are easily altered by subtle means; Their concentrations are of central importance to cellular activity; Applicable to human blood cells; In-vivo or in-vitro dose-response studies can be done; In-vivo tests would be suitable for standard setting and regulatory purposes.

Weaknesses: Time consuming; Tedious; Requires a lot of cells; Difficult to establish steady basal or control values; In-vivo exposures will require relatively large amounts of pollutant samples.

Status of Development: Being implemented.

Describe: Effects of NO_2, O_3, and NO are being tested for effects on Cyclic AMP and Cyclic GMP.

Applications: Air; Water; Food; Multimedia.

Samples: Pure Chemicals: Most chemicals. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related.

Duration: In-vitro exposure: 2 weeks/chemical for dose-response; In-vivo exposure: 4 weeks/chemical for dose-response.

Cost: In-vitro exposure: $5,000/chemical; In-vivo exposure: $10,000/chemical.

Interpretation: Detection of alteration in cellular hormone metabolism which is important to homeostasis.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: G. Hatch, U.S. EPA, HERL-RTP, Biomedical Research Branch, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Hatch, G.E., W.K. Nichols, and H.R. Hill. J. Immunol., 119:450-456, 1977.

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1141 TOXICITY OF AEROSOLIZED POLLUTANTS/ACUTE AND SUBACUTE

Biological Activity Detected: Toxicity.

Principle: Exposure begins with a relatively high concentration, which results in 100% mortality in 1 to 4 hours. The concentration is decreased by factors of 10 until no deaths occur. Performed in rats.

Endpoints: Qualitative: N/A. Quantitative: Deaths of animals being exposed.

Strengths: Non-ambiguous positive or negative results.

Weaknesses: For moderately or low toxic substances the determination of a LC50 may be impractical.

Status of Development: Being implemented.

Describe: The equipment required for aerosol generation in the respirable range is being evaluated.

Applications: Air.

Samples: Pure Chemicals: Pesticides. Complex Mixtures: N/A.

Duration: Variable, 1 week to 2 months.

Cost: $10,000 to $20,000/analysis.

Interpretation: A rating of the relative toxicity in mammalian species is obtained.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Toxic Effects Branch, Inhalation Toxicology Group.

Persons to Contact: J. Charles, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2696).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OPP; OTS.

References: 1) Hinners, R.G., J.K. Burkhart, and C.L. Punte. Arch. Envir. Hlth., 16:194-206, 1968.

1142 TOXICITY OF GASEOUS AND VAPOR PHASE/ACUTE AND SUBACUTE

Biological Activity Detected: Toxicity.

Principle: Exposure begins with a relatively high concentration which results in 100% mortality in 1 to 4 hours. The concentrations are decreased by factors of 10 until no deaths occur. Performed primarily in rats. Physiologic and biochemical parameters are measured.

Endpoints: Qualitative: N/A. Quantitative: Death of animals being exposed. Biochemical parameters, enzymes, and substrate levels are assayed for in surviving animals.

Strengths: Non-ambiguous positive or negative results.

Weaknesses: For moderately or low toxic substances the determination of an LC50 may be impractical.

Status of Development: Being implemented.

Describe: 5 chambers, 20 animals/chambers are in operation.

Applications: Air.

Samples: Pure Chemicals: Gases, Vaporizable liquids, Pesticides, Toxic substances in general. Complex Mixtures: N/A.

Duration: Variable, 1 week to 2 months.

Cost: $10,000 to $20,000/analysis.

Interpretation: A rating of the relative toxicity in mammalian species is obtained.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Toxic Effects Branch, Inhalation Toxicology Group.

Persons to Contact: J. Charles, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2696).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OPP; OTS.

References: 1) Drew, R.T., and S. Laskin. Methods of Animal Experimentation, Vol. 4. N.Y. Academic Press. pp. 1-41. 2) Hinners, R.G., J.K. Burkhart, and C.L. Punte. Arch. Envir. Hlth., 16:194-206, 1968.

1143 INHALATION LC50 TESTS

Biological Activity Detected: Toxicity.

Principle: Animals are exposed to different concentrations of the agent, and lethality is determined over a preset period of time.

Endpoints: Qualitative: N/A. Quantitative: Concentration required to cause death in 50% of the exposed animals.

Strengths: Provides initial data on toxicity.

Weaknesses: Cannot be used to determine subtle changes associated with low level exposure.

Status of Development: Validated.

Describe: Most chemicals and complex mixtures can be examined; extremely toxic or carcinogenic samples cannot be examined as HERL-CIN does not have the necessary facilities.

Applications: Multimedia.

Samples: Pure Chemicals: Most chemicals. Complex Mixtures: Ambient, Industrial; Energy Related; Transportation Related; Other.

Duration: 14 days/study.

Cost: $4,000 to $10,000, test dependent.

Interpretation: A rating of the relative toxicity in mammalian species is obtained.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Functional Pathology Branch; HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: W. Moore, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7431); D.E. Gardner, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: ORD.

References: 1) Moore, W., M. Malanchuk, W. Crocker, D. Hysell, A. Cohen, and J.S. Stara. Whole Body Retention in Rats of Different 191Pt Compounds Following Inhalation Exposures. Envir. Hlth. Perspec., 12:35, 1973. 2) Moore, W., J.S. Stara, W. Crocker, M. Malanchuk, and R. Iltis. Comparison of Cadmium Retention in Rats Following Different Routes of Administration. Envir. Res., 6:473, 1973.

1144 DEPOSITION AND CLEARANCE OF RADIOACTIVE MATERIALS FOLLOWING INHALATION EXPOSURE

Biological Activity Detected: Deposition; Translocation clearance.
Principle: Animals are exposed to radioactive aerosol for 15 to 30 minutes; then they are counted at various intervals of time. Tissues are also taken for analysis.
Endpoints: Qualitative: N/A. Quantitative: Data on clearance, translocation, distribution, and excretion as a factor of time.
Strengths: Provides metabolic parameters on the agent under study.
Weaknesses: Agents used are usually radioactive.
Status of Development: Validated.
Describe: N/A.
Applications: Multimedia.
Samples: Pure Chemicals: Radioactive chemicals. Complex Mixtures: N/A.
Duration: Variable, depending on the goals of the study.
Cost/sample: $3,000 to $5,000, depending on cost of compound.
Interpretation: A measure of the residence tissue in the lung and body tissues for the compound is obtained.
Level of Complexity: 3.
OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Functional Pathology Branch; HERL-RTP, Clinical Studies Division, Biomedical Research Branch.
Persons to Contact: W. Moore, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7431); D.E. Gardner, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711 (FTS 629-2531).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: ORD.
References: 1) Moore, W., D. Hysell, W. Crocker, and J.S. Stara. Biological Fate of ^{103}Pd in Rats Following Different Routes of Exposure. Envir. Res., 8:234, 1974.

1145 INTEGRATED SYSTEM: PULMONARY FUNCTION AND PULMONARY METABOLISM

Biological Activity Detected: Toxicity.

Principle: Structural changes in lung airways and parenchyma can be evaluated in-vivo by use of appropriate tests of pulmonary function. Concommitant metabolic changes can be estimated in-vitro.

Endpoints: Changes can be measured in intact animals. Qualitative and Quantitative: Changes in pulmonary mechanics, spirometry, and diffusion can be evaluated. Metabolic changes of lung tissue can be estimated in-vitro to establish dose-response relationships.

Strengths: Measurements of pulmonary function accomplished by non-invasive methods; In-vivo dose-response effects more applicable for standard-setting and regulatory purposes.

Weaknesses: Expensive equipment; Time consuming; Requires several pairs of hands; Some tests are still being validated; Parenchymal changes (i.e., development of pulmonary fibrosis, pulmonary emphysema or chronic bronchitis) are of great interest in terms of health effects, but they are usually chronic diseases.

Status of Development: Developmental; Being implemented; Validated. Describe: Different tests are in different stages of development. We are presently measuring lung volumes and capacities (TLL, VC, IC, FRC, RV, V_T), breathing frequency, minute ventilation, diffusing capacity for carbon monoxide, nitrogen washout, and quasi-static pressure volume relationships of the lung and chest wall. We are developing methods to measure dynamic compliance and resistance, maximum flow volume relationships, compliance characteristics of excised lungs, and the single breath oxygen test.

Applications: Air.

Samples: Pure Chemicals: Oxides of N and S, Pulmonary toxicants (e.g. Paraquat), Pesticides. Complex Mixtures: Ambient - NO_2, SO_2, O_3; Energy Related - particulates and NO_x, SO_x, O_3, organics, others; Transportation Related - diesel; Other - Toxic substances, Any compound which changes pulmonary physiology.

Duration: 3 month/dose-response of 1 chemical.

Cost: $30,000/dose-response of 1 chemical.

Interpretation: These tests are sensitive and can detect small changes in lung physiology.

Level of Complexity: 3 to 4.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: J.J. O'Neil, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2711/2531).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Koo, K.W., et al. Respiratory Mechanics in Normal Hamsters. J. Appl. Physiol., 40:936-942, 1976. 2) Diamond, L., and M. O'Donnell. Pulmonary Mechanics in Normal Rats. J. Appl. Physiol., 43:942-948, 1977. 3) Takezawa, J., F. Miller, and J.J. O'Neil. Single Breath Diffusing Capacity and Lung Volumes in Small Laboratory Animals. J. Appl. Physiol., 1979. In preparation.

1146 PULMONARY FUNCTION IN RATS

Biological Activity Detected: Toxicity.

Principle: Residual volume of the lung increases with many types of obstructive and destructive lung damage. The slope of the static compliance of the pressure-volume curve of the lungs increases with fibrosis and decreases with diseases such as emphysema that destroy alveolar tissue.

Endpoints: Qualitative: N/A. Quantitative: Residual volume (cc) and the slope of the static compliance curves.

Strengths: The measurements are sensitive indicators of lung volume; They are relatively easy to perform.

Weaknesses: The measurements are conducted with rats; Pulmonary anatomy and susceptibility of these animals may differ somewhat from man.

Status of Development: Validated.

Describe: Rats have shown large changes in residual volume and in the slope of the static compliance curve, after 1 to 14 days of exposure to either 0.75 or 1.0 ppm ozone. A manuscript describing these results is being prepared.

Applications: Air; Water; Food.

Samples: Pure Chemicals: Sulfates, Oxidants, Heavy metals.

Complex Mixtures: Ambient - air; Industrial - coal dust; Energy Related - stack gases; Transportation Related - diesel and gasoline exhaust; Other - asbestos.

Duration: 1 day to 30 days.

Cost/sample or chemical: $1,000/animal.

Interpretation: An increase in the slope of the static compliance curve and/or an increase in residual volume is indicative of either pulmonary edema or emphysema.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Functional Pathology Branch.

Persons to Contact: W.E. Pepelko, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7437).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) J. Appl. Physiol., 26:738-744, 1966. 2) Chest, 475-481, 1967.

1147 PULMONARY FUNCTION OF UNANESTHETIZED GUINEA PIGS

Biological Activity Detected: Toxicity.

Principle: The method allows for measurement of respiratory and cardiovascular response of an unanesthetized guinea pig to a controlled test atmosphere.

Endpoints: Qualitative: ECG. Quantitative: Airway resistance, lung compliance, tidal volume, minute volume, breathing rate, heart rate.

Strengths: This system is most suitable for 1 to 3 hour exposures to gases, aerosols, drug response, complex pollutants, etc.; Each animal serves as its own control during testing.

Weaknesses: The system can be used for measuring response to a long term exposure where chronic breathing damage is expected. However, there is wide variation between animals. So for reliable results either a dramatic response is needed or a very large number of animals.

Status of Development: Being implemented.

Describe: System has been used for catalytically altered auto exhaust study, sulfur dioxide exposure study, and diesel engine auto exhaust study. Results are presently being evaluated.

Applications: Air; Multimedia.

Samples: Pure Chemicals: Sulfur dioxide. Complex Mixtures: Transportation Related - catalytically altered car exhaust, diesel auto emissions.

Duration: 5 hours/animal.

Cost/sample or chemical: $500/sample. This refers only to the test animals needed.

Interpretation: Reliable results require dramatic response differences.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN.

Persons to Contact: M.J. Wiester, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7424).

Grant/Contract Laboratory Involved and Principal Investigators: In-house.

Program Office Support: OHEE.

References: 1) Amdur, M.O., and J. Mead. Mechanics of Respiration in Unanesthetized Guinea Pigs. Am. J. Physiol., 192:364, 1958.

1148 ARTERIAL BLOOD GAS MEASUREMENT IN CONSCIOUS RATS

Biological Activity Detected: Toxicity.
Principle: Pollutant inhalation resulting in lung damage can decrease the ability of the animal to oxygenate the blood and remove CO_2.
Endpoints: Qualitative: N/A. Quantitative: Arterial blood P_{O_2}, P_{CO_2}, pH, bicarbonate.
Strengths: A sensitive indicator of lung damage; An important parameter as adequate P_{O_2} levels are necessary to support life; A sample can be collected quickly and fairly easily.
Weaknesses: Rats are the only small laboratory animal to which this method can be adapted; Extrapolation of results from rats to humans may be subject to criticism; Each animal can be used only once.
Status of Development: Validated.
Describe: Arterial blood gas measurements were carried out after 1, 3, 7, and 14 days exposure to either 0.75 or 1.0 ppm ozone. Arterial blood P_{O_2} declined in proportion to lung damage. A manuscript is in preparation.
Applications: Air; Water; Food.
Samples: Pure Chemicals: Sulfates, Oxidants, Trace metals.
Complex Mixtures: Ambient - air; Industrial - coal and rock dust; Energy Related - stack gas emission; Transportation Related - auto and diesel emission; Other - food and water born pollutants such as Paraquat.
Duration: 24 hours to 30 days.
Cost/sample or chemical: $200/animal.
Interpretation: A decrease in arterial P_{O_2} or an increase in P_{CO_2} indicates lung damage. The type of damage is not specified
Level of Complexity: 3.
OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Functional Pathology Branch.
Persons to Contact: W.E. Pepelko, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7437).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OHEE.
References: 1) J. Appl. Physiol., 38:581-587, 1975. 2) Ann. Rev. Pharmacol. and Toxicol., 16:465-486, 1976. 3) Am. Rev. Resp. Dis., 113:531-559, 1976.

1149 INFECTIVITY MODEL

Biological Activity Detected: Toxicity.

Principle: Inhalation of a variety of gases and particulates has been shown to increase susceptibility to infectious pulmonary disease. Evaluation of the infectivity model used for these tests indicates that the model reflects the effects of a pollutant on a number of host defense systems, thereby increasing its sensitivity for detecting effects.

Endpoints: Qualitative: N/A. Quantitative: Mortality from laboratory-induced bacterial pneumonia is measured.

Strengths: The model is established for inhalation toxicology; Prior work has shown its exquisite sensitivity; The test is rapid; A battery of related follow-up tests are available; Whole animal inhalation exposures and dose-response studies are more directly applicable to standard setting and regulatory action.

Weaknesses: Relatively large amounts of pollutant sample are required for inhalation studies.

Status of Development: Validated.

Describe: The model has been successfully used for O_3, NO_2, Cd, Ni, sulfates and pollutant combinations. Even though a great potential exists it has not been used for screening purposes.

Applications: Air.

Samples: Pure Chemicals: Any chemical likely to reach gaseous exchange areas of the lung. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other - any other particulate, gas or combination of same.

Duration: 8 weeks/dose-response of 1 chemical.

Cost: $30,000 to $35,000/dose-response of 1 chemical.

Interpretation: Estimation of enhanced susceptibility to infectious diseases due to pollutant.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: D.E. Gardner, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: IIT Research Institute, 10 West 35th St., Chicago, IL 60616, R. Ehrlich; Northrop Services, Inc., P.O. Box 12313, Research Triangle Park, NC 27709, B. Adkins.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Coffin, D.L., and D.E. Gardner. Ann. Occup. Hyg., 15:219-234, 1972. 2) Coffin, D.L., et al. Envir. Hlth. Perspect., 13:11-15, 1976. 3) Ehrlich, R. Bacteriol. Rev., 30:604-614, 1966. 4) Ehrlich, R., et al. Internat. Conf. Photo. Oxid. Pollut. and Its Control, Proc. Vol I, EPA-600/3-77-001a, 1977. pp. 565-574.

11410 IN-VIVO ALVEOLAR MACROPHAGE CYTOTOXICITY

Biological Activity Detected: Toxicity.

Principle: Cytotoxic effects of inhalation of environmental chemicals will be measured using isolated alveolar macrophages. Any alteration in these cells could increase the potential risk of respiratory infections.

Endpoints: Qualitative: N/A. Quantitative: The following measurements can be made: viability, phagocytic functioning, bacteriocidal activity, enzymatic profile, morphology and other biochemical parameters.

Strengths: A sensitive indicator of cytotoxicity using an in-vivo model system; Data generated quickly, which can be used to validate in-vitro cytotoxicity testing; Can serve as criteria for standard setting and regulatory purposes.

Weaknesses: Requires a substantial quantity of the test chemicals for generation of aerosols.

Status of Development: Validated.

Describe: The model has been successfully used for O_3, NO_2, Cd, Ni, Mn, cigarette smoke, and other metals.

Applications: Air.

Samples: Pure Chemicals: Particulates, Gases, Mists, Any chemical likely to reach gaseous exchange areas of the lung. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related.

Duration: 8 weeks/dose-response of 1 chemical.

Cost: $30,000/dose-response of 1 chemical.

Interpretation: Depending upon the particular endpoint, alteration would indicate enhanced susceptibility to infectious disease or potential for alteration of lung tissue.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-RTP, Clinical Studies Division, Biomedical Research Branch.

Persons to Contact: D.E. Gardner, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2531).

Grant/Contract Laboratory Involved and Principal Investigators: IIT Research Institute, 10 West 35th St., Chicago, IL 60616, C. Aranyi; U. of California, Davis, CA 95616, E. Goldstein; Southwest Research Institute, San Antonio, Texas 78284, E. Gause.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Gardner, D.E., et al. J. Bacteriol., 98:1041-1043, 1969. 2) Hurst, D.J., et al. J. Reticuloendothel. Soc., 8:288-300, 1970. 3) Bingham, E. Arch. Envir. Hlth., 25:406-414, 1972. 4) Gardner, D.E. Thesis, U. of Cincinnati, 1971. 5) Warshauer, D., et al. J. Lab. Clin. Med., 83:228-240, 1974. 6) Kass, E.H., et al. Bacteriol. Rev., 30:488-497, 1966. 7) Kim, M., et al. J. Infect. Dis., 133:310-320, 1976.

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1151 INTEGRATED SYSTEM: NEUROBEHAVIORAL TOXICOLOGICAL ASSESSMENT

Biological Activity Detected: Toxicity.

Principle: A battery of tests are being implemented which evaluate various aspects of CNS functions.

Endpoints: Qualitative: Evaluate CNS Function. Qualitative: These include: locomotor activity, neuromotor function, CNS excitability, learning and memory.

Strengths: Provides rapid and sensitive broad spectrum evaluation of CNS function.

Weaknesses: Requires skilled personnel; Real possibility of false negatives.

Status of Development: Validated.

Describe: This battery has been implemented and is currently being validated using known neurotoxins.

Applications: Multimedia.

Samples: Pure Chemicals: Heavy metal, Pesticides, Nonionizing radifilm. Complex Mixtures: N/A.

Duration: Test: 2 weeks, assuming acute exposure; Analysis: 3 weeks.

Cost: $2,000/sample, assuming acute exposure.

Interpretation: The profile of change in the various functional tests provide information on neurotoxicity of test compounds.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Biology Division, Neurobiology Branch.

Persons to Contact: L. Reiter, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2671).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Reiter, L., et al. Residential Maze. Envir. Hlth. Perspect., 12:119-123, 1975. 2) Archer, J. Anim. Behav., 21:205-235, 1973. 3) Dunham, N.W., and T.S. Miya. Roto Rod. J. of Amer. Pharmaceu. Asso., 46(3):208-209, 1957. 4) Gait, S., R. Rushton, and H. Stellberg. Anim. Behav. and Drug Action, 207-223, 1964. 5) Tremors, R.R., G.K. Chalmers, and W. Yim. Proc. Soc. Exp. Biol. Med., 109:202-205, 1962. 6) Hornston, M. Startle Reflex. Physio. and Rev., 3:839-844, 1968. 7) Reiter, L., et al. Passive Avoidance Test. Toxicol. and Appl. Pharm., 25:582-588, 1973. 8) Miczek, K., and H. Barr, II. Social Behavior. Behav. Pharmacol., 176-257, 1976.

1152 INTEGRATED SYSTEM: THE EFFECTS OF SELECTED ORGANIC CONTAMINANTS IN DRINKING WATER ON THE FUNCTIONS OF THE REPRODUCTIVE, NERVOUS, AND IMMUNE SYSTEMS

Biological Activity Detected: Reproductive, immuno-, neuro-, and behavioral toxicity; Mutagenicity.

Principle: Determine effects of experimental exposure to certain selected organic contaminants on: 1) Immune system and host resistance capabilities; 2) Neurochemical processes and dynamics in the brain; 3) Behavior; 4) Male reproductive function (including dominant lethal mutagenicity assay); 5) General toxicity endpoints (lethality, body-organ weights, hematology, etc.).

Endpoints: Qualitative: N/A. Quantitative: 1) Immune response and host resistance: Humoral - serum antibody production to S. aureus; Cell mediated - response to C. parvum; RES activity - global phagocyt. index (vasc. clearance) and tissue distribution of ^{14}C S. aureus; Susceptibility to pathogens (bact., virus, fungus) and transplant tumor; 2) Brain neurochemistry and dynamics: In-vivo and in-vitro (synaptosome) systems; Endogenous levels, uptake, release, metabolism...in Norepinephrine, dopamine, serotonin and AcCh systems; 3) Behavioral toxicology: Operant behavior - scheduled and learned performance; Learning ability; Behavior development; Other - elem. screen, spont. mot. activ., visual, swim, maze, etc; 4) Reproductive performance and dom. lethal mutagen. asso.: Antifertility, reversibility, mutagenic potential, mode of action, penetration of BT barrier (in-vivo); Penetration mechanism, spermatic nucleo/protein synth. spermatic uptake (in-vitro); 5) Preliminary and range finding shorter term toxicology: Lethality, body/organ weights, hematology, etc.

Strengths: Provides much information on several toxicologic aspect areas in coordinated manner; Readiness reduces time turnaround.

Weaknesses: Expensive.

Status of Development: Being implemented.

Describe: The tests are being implemented. Some are still developmental, but most are validated. Current work applicable to and aimed at drinking water contaminants, but techniques and inferences applicable to other media.

Applications: Water.

Samples: Pure Chemicals: Trihalomethanes; Benzenes; Ethers; Phenyls; Pesticides; PCB; Dioxin; 2, 4-DNT; (Many chemicals, but not all are under test by all the test systems under present project). Complex Mixtures: Not now under test but could be implemented.

Duration: Existing grants, about 2 years, covering multiple chemical; Individual tests involve short (1 to several days) to long-term (3 months) exposures.

Cost/sample or chemical: Approx. $120,000 for the entire integrated multidisciplinary workup involving multiple dose levels and exposure periods lasting up to a year for completion of all phases. However, application of selected portions, reduced exposures or fewer dose levels, etc., would be less expensive.

1152 INTEGRATED SYSTEM: THE EFFECTS OF SELECTED ORGANIC CONTAMINANTS IN DRINKING WATER ON THE FUNCTIONS OF THE REPRODUCTIVE, NERVOUS, AND IMMUNE SYSTEMS (continued)

Interpretation: Toxicity interpreted in terms of potential human health hazards.

Level of Complexity: Complexity levels range from 3 to 5.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicologic Assessment Branch.

Persons to Contact: K.I. Campbell, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7481).

Grant/Contract Laboratory Involved and Principal Investigators: Medical College of Virginia, Richmond, VA 23298: J.F. Borzelleca, Project Manager (Coordinator) and Reproduction Studies; A.E. Munson, Immune Systems, etc.; R.L. Balster, Behavioral Toxicology; W.L. Dewey, Neurochemistry.

Program Office Support: ORD.

References: 1) Grant document and report: R804701. 2) Grant document and report: R804290. 3) Deichmann, W., et al. Toxicol. and Appl. Pharmacol., 5:201, 1963. 4) Ball, H. J. Nat. Can. Inst., 44:1070, 1966. 5) Szakol, L., and H. Hanna. Nat. Can. Inst. Monographo., 35:173, 1972 6) For additional related references, contact investigator.

1153 COMPUTER AUTOMATED ANALYSIS OF PATTERNED BEHAVIOR IN THE PRIMATE

Biological Activity Detected: Toxicity.

Principle: Animal behavior is structured (patterned) and chemicals which affect CNS function will disrupt this patterned behavior.

Endpoints: Using closed-circuit TV and on-line computing, various components of primate behavior are defined and measured for frequency, duration, and sequences. Qualitative: N/A. Quantitative: Frequency of various motor items in experimental period; Duration of various motor items per occurrence; Sequencing of behavior.

Strengths: Should provide sensitive index of neurotoxicity which is fully automated and applicable to a wide range of pollutant testing; Also no pre-training is required.

Weaknesses: Expensive; Specialized equipment is required.

Status of Development: Developmental.

Describe: System has been developed and is being tested with known psychoactive drugs.

Applications: Multimedia.

Samples: Pure Chemicals: Heavy metals. Complex Mixtures: N/A.

Duration: Test: 1 week, assuming acute exposure; Analysis: 2 weeks.

Cost: $200/subject.

Interpretation: Changes in frequency, duration, or patterning of behavior are indicative of neurotoxicity.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Biology Division, Neurobiology Branch.

Persons to Contact: L. Reiter, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2671).

Grant/Contract Laboratory Involved and Principal Investigators: Iowa State U., Ames, IA 50010, W.E. Lloyde.

Program Office Support: OEMI.

References: 1) Norton, S. Physiol. Behav., 11:181-186, 1973. 2) Norton, S. J. Theoret. Biol., 21:229-243, 1962.

1154 COMPUTER AUTOMATED ANALYSIS OF LEARNING AND MEMORY IN THE PRIMATE

Biological Activity Detected: Toxicity.
Principle: Chemicals affecting CNS function may interfere with the learning process. The fact that primates rely heavily on the visual sensory modality is utilized to determine pollutant effects on visual discrimination learning.
Endpoints: Qualitative: N/A. Quantitative: Two choice non-spatial visual discrimination tasks examine animals' ability to distinguish between different visual patterns; Delayed response task: evaluates memory coordination utilizing visual stimuli.
Strengths: Provides system index of chemical effects on learning and memory; Provides information on behavioral effects in primates.
Weaknesses: Expensive; Requires skilled personnel to perform test; Used only for toxicity testing of pure compounds when specific information on primates is required.
Status of Development: Developmental.
Describe: System has been developed and is being tested with psycho-active drugs.
Applications: Multimedia.
Samples: Pure Chemicals: Heavy metals. Complex Mixtures: N/A.
Duration: Test: 6 weeks, assuming acute exposure; Analysis: 2 weeks.
Cost: $200/subject.
Interpretation: Alterations in performace are indicative of neurotoxicity.
Level of Complexity: 3.
OHEE Laboratory Involved: HERL-RTP, Environmental Biology Division, Neurobiology Branch.
Persons to Contact: L. Reiter, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2671).
Grant/Contract Laboratory Involved and Principal Investigators: Iowa State U., Ames, IA 50010, W.E. Lloyde.
Program Office Support: OEMI.
References: 1) Fletcher, H.J. The Delayed-Response Problem. In: Behavior of Nonhuman Primates, A.M. Schrier, H.F. Harlow, and F. Stollnitz, eds. Academic Press, New York, 1965. pp. 129-165. 2) Miles, R.C. Discrimination-Learning Sets. In: Behavior of Nonhuman Primates, A.M. Schrier, H.F. Harlow, and F. Stollnitz, eds. Academic Press, New York, 1965. pp. 53-54.

1155 INTEGRATED SYSTEM: BEHAVIORAL ANALYSIS OF RATS — DEVELOPMENTAL, LOCOMOTOR, EXPLORATORY, AND LEARNING BEHAVIOR

Biological Activity Detected: Toxicity; Behavioral.

Principle: Various levels of lead are known to affect the maturation of energy metabolism in the cerebral cortex of rats. Studies have shown that delays in the development of the nervous system may affect behavioral responses observable in both the young and adult animal. Specific affects need to be tested on the above listed indices.

Endpoints: Qualitative: Expression of learned behavior through use of a water T-Maze - percentage of correct trials and amount of time to reach criteria; Expression of locomotor activity and exploratory behavior through the use of a Berylene Box - primarily a measurement of frequency and duration spent on each parameter; Expression of developmental behavior through locomotor activity and use of ultrasonics - the measurement of general activity patterns and monitoring of communication abilities dependent upon development. Quantitative: Maze activity; Benylene box; Generalized and specific locomotor activity; Frequency and duration of ultrasonic vocalizations.

Strengths: Should provide sensitive behavioral tests which can then be correlated with physiological data collected in previous studies.

Weaknesses: Requires specialized instrumentation for testing; Time-consuming to run the battery of tests for each designated dosage level.

Status of Development: Being implemented.

Describe: Most of the instrumentation has been constructed and subjects are being treated with designated lead doses.

Applications: Water.

Samples: Pure Chemicals: Trace metals, Organic chemicals.

Complex Mixtures: Industrial; Energy Related.

Duration: 1 to 3 months.

Cost: Not yet established.

Interpretation: Altered physiological or neurological function as a result of treatment chemical would indicate toxicity.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OWHM.

References: 1) Reiter, L.W., et al. Envir. Hlth. Perspect., 12:119-123, 1975. 2) Sobotka, T.J., et al. Toxicol., 5:175-191, 1975. 3) Brown, D.R. Toxicol. Appl. Pharmacol., 32:628-637, 1975. 4) Padich, R., et al. Pharmacol. Biochem. Behav., 6:371-375, 1977. 5) Fox, D.A., et al. Toxicol. Appl. Pharmacol., 40:449-461, 1977. 6) Robbins, T., et al. Psychopharmacologia, 28:155-164, 1972. 7) Amsel, A., et al. Science, 197:786-788, 1977.

1156 INTEGRATED SYSTEM: INSTRUMENTAL METHODS OF DETECTING FUNCTIONAL AND METABOLIC DAMAGE TO THE NERVOUS SYSTEM

Biological Activity Detected: Toxicity.

Principle: Increased functional activity of a tissue requires energy. Consequently, if a tissue's functional activity is stimulated, ATP is hydrolyzed to ADP and P_i, which in turn stimulates oxidation of substrate and resynthesis of ATP. These metabolic changes may be observed as increases in oxygen consumption, substrate utilization, or as metabolic transients induced in the electron carriers directly in tissues, in-vitro. The kinetics of these metabolic responses to stimulation have been shown sensitive to a wide variety of chemical agents with varying mechanisms of action with both in-vitro and in-vivo treatments.

Endpoints: To this point in time, an endpoint has only been developed for brain tissue. Responses are measured in response to electrical pulses (10 s) or elevation in K concentrations (3 to 30 mM). Qualitative: N/A. Quantitative: Transient redox changes in NAD(P)H, fp, cyt a, b, c.; Substrate utilization; Oxygen consumption; Lactic acid output; Neurotransmitter release; Amino acid metabolism.

Strengths: In-vitro results may be directly confirmed in-vivo with the same parameters; Applicable to a wide variety of mechanisms; Applicable to all aerobic tissues; Involves measurement of the kinetics of going from a resting to an excited state rather than the steady state greatly increasing sensitivity; Applicable to very small tissue samplers (2 to 3 mg).

Weaknesses: Does not lend itself to immediate identification of mechanisms unless there is a direct effect on energy metabolism proper.

Status of Development: Validated.

Describe: The test system has been validated with a wide variety of inhibitors of energy metabolism and membrane active compounds. In-vitro and in-vivo treatments with lead, methyl mercury, and alkylation compounds indicate equivalent or more sensitive measures of effect than other parameters which have been applied to these problems.

Applications: Multimedia.

Samples: Pure Chemicals: All classes. Complex Mixtures: Industrial; Energy Related; Transportation Related.

Duration: 2 weeks to 3 months.

Cost: Not yet established.

Interpretation: This is a general system for determining neurotoxicity. It is capable of detecting non-specific damage to a variety of systems (e.g., decreased membrane excitability, altered responses to neurotransmitters, direct effects on energy metabolism). It has also been used to detect delays in brain development, which were subsequently confirmed by morphologic and behavioral methods.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

1156 INTEGRATED SYSTEM: INSTRUMENTAL METHODS OF DETECTING FUNCTIONAL AND METABOLIC DAMAGE TO THE NERVOUS SYSTEM (continued)

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213).

Grant/Contract Laboratory Involved and Principal Investigators: In-house.

Program Office Support: OHEE.

References: 1) Bull, R.J., and A.J. Trevor. J. Neurochem., 19:999-1009, 1972. 2) Bull, R.J., and A.J. Trevor. J. Neurochem., 19: 1011-1022, 1972. 3) Cummins, J.T., and R. Bull. Biochem. Biophys. Acta, 253:29-38, 1971. 4) Bull, R.J., and J.T. Cummins. J. Neurochem., 21:923-937, 1973. 5) Bull, R.J., and S.D. Lutkenhoff. Neuropharmacol., 14:351-359, 1975. 6) Bull, R.J. J. Neurochem., 26:149-156, 1976. 7) Bull, R.J., P.M. Stanaszek, J.J. O'Neill, and S.D. Lutkenhoff. Envir. Hlth. Perspect., 12: 89-95, 1975.

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1211 SALMONELLA TYPHIMURIUM (AMES)

Biological Activity Detected: Mutagenicity; Presumptive oncogenicity.

Principle: Histidine dependent strains of Salmonella genetically engineered to increase their sensitivity and specificity, are exposed to a test substance in the presence of mammalian metabolic activating enzymes. The formation of bacterial colonies in a histidine-free medium after treatment is considered a criteria of the effectiveness of a test substance as a mutagen.

Endpoints: Qualitative: Spot test and plate incorporation test are considered to be qualitative in nature. In spot tests no attempt is made to count the number of colonies per plate. In plate incorporation the number of colonies are counted but not expressed as a fraction of survival. Quantitative: The induced mutational frequencies may be expressed on the basis of units of test material and survival in suspension.

Strengths: Genetically well-characterized system; Rapid; Inexpensive; Well validated as a test for gene mutation; Works well with in-vitro metabolizing microsome fractions; Can be used as indicator organism in host-mediated assays.

Weaknesses: Reverse mutation assay requiring several strains to permit detection of a broad spectrum of compounds; Requires metabolic activation; Lacks pharmacological relevance; Prokaryotic organization of genetic material.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: All major classes of chemicals except metals and hormones. Complex Mixtures: Ambient - air particulates, drinking water, and water concentrates; Industrial - effluents; Energy Related - alternate effluents, shale; Transportation Related - auto/truck fuels; Other - human body fluids, extracts from crops treated with sludge.

Duration: 3 weeks/study.

Cost: $300 to $650/compound for plate test; $1,000 to $1,200/compound for suspension test.

Interpretation: The growth of colonies in a histidine-deficient medium indicates genetic alteration.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section; HERL-CIN, Field Studies Division, Toxicological Assessment Branch; ERL-GB; National Center for Toxicological Research, Division of Mutagenesis, Somatic Cell Section.

Persons to Contact: J.L. Huisingh, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2948); L. Claxton, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2942); M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); J.P. Bercz, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7432); H.R. Pahren, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7217); L.J. McCabe, U.S. EPA, HERL-CIN, 26 W. St. Clair St.,

1211 SALMONELLA TYPHIMURIUM (AMES) (continued)

Persons to Contact (continued): Cincinnati, OH 45268, (FTS 684-7211); J.F. Stara, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7407); N. Richards, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011); E. Lazear, NCTR, Jefferson, AR 72079, (FTS 740-4573); D.A. Casciano, NCTR, Jefferson, AR 72079, (FTS 740-4495).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA, V.F. Simmons; Litton Biometics, Inc., Nicholson Lane, Kensington, MD, D.T. Brusick; U. of Cincinnati Medical Center, J. Loper; Louisiana State U. Medical School, W. Pelon; U. of Missouri, Columbia, MO, C. Marianseld; U. of West Florida, J. Bazlis; U. of Texas, Medical Branch, Galveston, TX 77550, M. Legator.

Program Office Support: OHEE; OPP; OEMI; OTS.

References: 1) Ames, B.N., et al. Mutation Res., 31:347-364, 1975. 2) McCann, et al. Proc. Natl. Acad. Sci., 70:782-786, 1975. 3) McCann, and B.N. Ames. Proc. Natl. Acad. Sci., 73:950-954, 1976.

1212 ESCHERICHIA COLI (WP2)

Biological Activity Detected: Mutagenicity.

Principle: Tryptophan dependent strains of Escherichia coli genetically engineered to increase their sensitivity and specificity, are exposed to a test substance in the presence of mammalian metabolic activating enzymes. The formation of bacterial colonies in a tryptophan-free medium after treatment indicates the effectiveness of a test substance as a mutagen.

Endpoints: Qualitative: Growth in a tryptophan-free medium.
Quantitative: N/A.

Strengths: Rapid; Inexpensive; Well validated test for gene mutation; Works well with in-vitro metabolizing microsomal enzymes; Can be used as indicator organism in host-mediated assays.

Weaknesses: Reverse mutation assay requiring several strains to permit detection of a broad spectrum of compounds; Requires metabolic activation; Lacks pharmacological relevance; Prokaryotic organization of genetic material; Not as well characterized nor as sensitive as Salmonella/microsome assay (1211). It detects only base pair substitutions.

Status of Development: Validated.
Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: All major classes of chemicals except metals and hormones. Complex Mixtures: Ambient - air particulates, drinking water, and water concentrates; Industrial - effluents; Energy Related - alternate effluents, shale; Transportation Related - auto/truck fuels; Other - human body fluids, extracts from crops treated with sludge.

Duration: 3 weeks.

Cost: $450/chemical.

Interpretation: The growth of tester strains in a tryptophan free medium after treatment with a test substance indicates mutation.

Level of Complexity: 1.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); L. Claxton, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2942); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); J.L. Huisingh, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2948).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, V.F. Simmons.

Program Office Support: OHEE; OPP.

References: 1) Bridges, B.A. Lab Practice, 21:413-416, 1972.
2) Bridges, B.A., et al. Mutation Res., 19:295-303, 1973.
3) Bridges, B.A., et al. Chem/Biol. Interactions, 5:77-84, 1972.

1213 SACCHAROMYCES CEREVISIAE, SCHIZOSACCHAROMYCES POMBE, FORWARD AND REVERSE MUTATION

Biological Activity Detected: Mutagenicity.

Principle: Gene mutation is detected by loss of function resulting in nutritional requirements or resistance to toxic chemicals and shift in color of cell colonies.

Endpoints: Qualitative: Growth in a selective medium; Change in colony pigmentation. Quantitative: Mutation frequencies may be adjusted for cytotoxic effects.

Strengths: Both forward and reverse mutation can be studied; Eukaryotic organization of genetic material; Fast; Relatively inexpensive; Cells can be cultured as haploids; Fairly wide spectrum of genetic events can be scored; Can be used as indicator organism in host-mediated assays.

Weaknesses: Requires exogenous metabolic activation which has not worked well with yeast systems; Lacks pharmacological relevance; Chromosomes are too small to permit direct cytological observation; Relatively insensitive to some chemicals.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents, Halogenated hydrocarbons, Polycyclics, Carbamates. Complex Mixtures: Ambient - water concentrated; Other - extracts from crops treated with sludge, human body fluids.

Duration: 3 weeks.

Cost: $400 to $700/compound, depending on the test.

Interpretation: The appearance of pigmented colonies and growth in selective mutation after treatment with test compound indicates mutation.

Level of Complexity: I.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); J.P. Bercz, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7432); H.R. Pahren, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7217).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, V.F. Simmons.

Program Office Support: OHEE; OPP; OEMI.

References: 1) Zimmermann, F.K. In: Chemical Mutagens: Principles and Methods for Their Detection. Vol. 3. A. Hollaender, ed. Plenum Press., NY, 1973. pp. 209-239. 2) Parry, J.M. Mutation Res., 46(3):165-176, 1977. 3) Brusick, D.J., and V.W. Mayer. Envir. Hlth. Perspect., 6:83-96.

Biological Activity Detected: Mutagenicity.

Principle: Promutagens which need mammalian metabolic activation are biotransformed in the intact animal and are tested for mutagenic activity in Salmonella, yeast, and Chinese hamster ovary cell test systems.

Endpoints: Qualitative: Appearance of revertant colonies in a selective medium. Quantitative: The number of prototrophic colonies in a histidine deficient medium, adjusted for cytotoxic effects of the test chemical.

Strengths: Combines in-vivo metabolic activation with the in-vitro microbial test system.

Weaknesses: Limited number of bacteria exposed; Recovery of bacteria is problematic; Exposure time has not been standardized; Difficult to quantitate the response.

Status of Development: Being implemented.

Describe: The basic experimental procedure for pure chemicals has been established. But protocol for testing for mixtures has not been developed. Furthermore, the test system needs validation by testing a wide variety of chemicals.

Applications: Multimedia.

Samples: Pure Chemicals: Mycotoxins, Nitrosamines, Aromatic amines, Aromatic hydrocarbons. Complex Mixtures: Not yet tested.

Duration: 4 weeks.

Cost: $1,000 to $1,200/chemical.

Interpretation: The positive response indicates the ability of the test chemical to be transformed into reactive electrophile by the intact mammalian metabolic activation system.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch; HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); L. Claxton, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2942); J.F. Stara; U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7407).

Grant/Contract Laboratory Involved and Principal Investigators:
U. of Texas, Medical Branch, Galveston, TX 77550, M. Legator.
Stanford Research Institute, Menlo Park, CA 94205, V.F. Simmons.

Program Office Support: OHEE; OPP; OEMI.

References: 1) Legator, M., et al. Mutation Res. 26:456, 1974.
2) Legator, M., et al. In: Chemical Mutagens: Principles and Methods for Their Detection. Vol. 4. A. Hollander, ed. Plenum Press, NY, 1976. pp. 171-190.

1215 BACTERIAL PLASMIDS

Biological Activity Detected: Mutagenicity.

Principle: Purified plasmid DNA is exposed to potential mutagen in a cell-free system and is then analyzed for ability to infect a host bacterium.

Endpoints: Qualitative: Ability of treated plasmid DNA to infect bacterial cells. Quantitative: The number of colonies produced per unit of test materials.

Strengths: May avoid problems of extreme cytotoxicity of many chemicals since the exposure is in a cell-free system; Rapid; Economical.

Weaknesses: Problems with exposing purified DNA; Still early in developmental stage; Requires exogenous metabolic activation systems.

Status of Development: Developmental.

Describe: Initial pilot work with MNNG is encouraging.

Applications: Multimedia.

Samples: Pure Chemicals: Organics. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other.

Duration: 1 to 2 days.

Cost: Undetermined.

Interpretation: Loss of infectivity suggests damage to DNA.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

Persons to Contact: N. Clarke, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7411); B. Daniel, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7482).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: Not yet available.

1216 MOUSE LYMPHOMA (L5178Y)

Biological Activity Detected: Mutagenicity.

Principle: Forward mutation from thymidine kinase competency ($TK^{+/-}$) to thymidine kinase incompetency ($TK^{-/-}$), resulting in resistance to the base analogs BUdR or TFT.

Endpoints: Gene mutation. Qualitative: Formation of cell colonies in a selective medium. Quantitative: Induced mutation frequencies are based on cell survival and cloning efficiency.

Strengths: Both forward and reverse mutation can be measured; Cell will grow in suspension culture; Cells have short generation time; Cells have stable, near-diploid chromosome number; High plating efficiency; High recovery of mutant cells.

Weaknesses: Mutation measured at a single locus; Requires metabolic activation; Requires additional validation; Lacks pharmacological relevance; PPLO contamination is a serious problem and the cells must be continuously monitored to ensure that they are PPLO-free.

Status of Development: Being implemented.

Describe: Problems of expression time and metabolic activation have yet to be resolved. This bioassay has been contracted out for validation by the National Cancer Institute.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents, Halogenated hydrocarbons, Inorganic derivatives, N-Nitroso compounds, Metals, Mycotoxins. Complex Mixtures: Ambient; Industrial.

Duration: 3 weeks.

Cost: $3,000/compound.

Interpretation: Growth of heterozygous thymidine competent cells in a medium containing TFT or BUdR suggests mutation.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); M.M. Brown, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, A. Mitchell.

Program Office Support: OHEE; OPP; OTS.

References: 1) Clive, D., and J.F.S. Spector. Laboratory Procedure for Assessing Specific Locus Mutations at the TK Locus in Cultured L5178Y Mouse Lymphoma Cells. Mutation Res., 31:17-29, 1975.

1217 CHINESE HAMSTER OVARY CELLS (CHO) DRUG RESISTANCE

Biological Activity Detected: Mutagenicity; Presumptive oncogenicity.

Principle: Forward mutation assay measuring drug resistance at hypoxanthine-guanine-phosphoribolyltransferase (HGPRT) locus. The HGPRT competent cells in presence of mammalian metabolic activation enzymes are exposed to a test substance. Induced frequency of HGPRT deficiency is determined using a selective medium containing base analogue 8-azaguanine (8 AZ) or 6-thioguanine (6 TH).

Endpoints: Gene mutation. Qualitative: Formation of cell colonies in a selective medium. Quantitative: induced mutation frequencies are based on cell survival and cloning efficiency.

Strengths: Mammalian organization of genetic material; Forward mutation assay; Fast generation time; Stable karyotype; Easy to culture; May be particularly well-suited to quantitation as HGPRT locus mutants may not show replicative advantages/disadvantages over wild-type cells under nonselective conditions.

Weaknesses: Mutation measured at a single locus; Requires metabolic activation; Needs additional validation; Lacks pharmacological relevance; Fairly high spontaneous mutation rate; Long optimal expression period (7 days).

Status of Development: Being implemented.

Describe: Basic system with metabolic activation has been described. Several compounds representing diverse classes of chemicals have been tested. However, additional chemicals by at least two laboratories have to be tested before it could be considered as validated.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents, Nitrosamines, Organics, PNA's, Metallic compounds. Complex Mixtures: Not yet determined.

Duration: 3 weeks/compound.

Cost: $2,000 to $3,000/compound, including dose-response.

Interpretation: Growth of HGPRT competent cells in a medium containing 8 AZ suggests mutation.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch; National Center for Toxicological Research, Division of Mutagenesis, Somatic Cell Section.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); D.A. Casciano, NCTR, Jefferson, AR 27079, (FTS 740-4495).

Grant/Contract Laboratory Involved and Principal Investigators:
Oak Ridge National Laboratory, Biology Division, P.O. Box Y,
Oak Ridge, TN 37830, A. Hsie.

Program Office Support: OHEE.

References: 1) O'Neill, P.J., et al. Mutation Res., 45:91-101, 1977.
2) O'Neill, P.J., et al. Mutation Res., 45:103-109, 1977.

1218 CHINESE HAMSTER CELLS (CHO) NUTRITIONAL COMPETENCY

Biological Activity Detected: Mutagenicity.

Principle: Isolation of auxotrophic mutants using 5-bromodeoxyuridine (BUdR) and visible light as selective agents.

Endpoints: Gene and chromosomal mutation. Qualitative: Formation of auxotrophic cell colonies. Quantitative: Can be used to determine induced mutation frequencies based on cell survival and cloning efficiency.

Strengths: System is clean (survivors are either auxotrophs or they are not); Several loci are available for detection of genetic changes; Forward mutation assay with very low spontaneous mutation frequencies at available loci; Test populations can be easily freed of spontaneous auxotrophs by growing cells three days in minimal medium; Mammalian system in terms of organization of genetic materials.

Weaknesses: Assay very insensitive (95% of auxotrophs lost during selection to effects of starvation); Mutant identification is tedious and not amenable to screening; Experiments require five weeks to complete; Requires metabolic activation; Requires validation; Lacks pharmacological relevance; Nutritional mutants appear to be at a replicative disadvantage in mixed populations under nonselective conditions.

Status of Development: Developmental.

Describe: Improvement of technique is necessary for increased sensitivity. Also necessary is the addition of an in-vitro metabolic activation.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating esters, Base analogs, Nitrosamines, Acridine, Mustards, Heavy metals, Hydroxylamine. Complex Mixtures: Energy Related - petroleum oil extracts; Transportation Related - jet fuel extracts; Other - ultraviolet radiation, x-rays.

Duration: 5 weeks.

Cost: $2,000/assay.

Interpretation: Test agents inducing significant numbers of auxotrophs may be regarded as potential mutagens/carcinogens for animals and man.

Level of Complexity: 4 to 5.

OHEE Laboratory Involved: ERL-NAR, Toxicology Branch, Genetic Toxicology Team.

Persons to Contact: A.R. Malcolm, U.S. EPA, ERL-NAR, South Ferry Rd., Narrangansett, RI 02882, (FTS 838-4843 X247 or X238).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Kao, F.T., and T.T. Puck. Induction and Isolation of Auxotrophic Mutants in Mammalian Cells. In: Methods in Cell Biology. Vol. 3. D. Prescott, ed. Academic Press, NY, 1974. pp. 23-39.

1219 CHINESE HAMSTER LUNG CELLS (V79)

Biological Activity Detected: Mutagenicity.

Principle: Forward mutation assay measuring drug resistance at hypoxanthine-guanine-phosphoribolyltransferase (HGPRT) locus. The HGPRT competent cells in presence of mammalian metabolic activation enzymes are exposed to a test substance. Induced frequency of HGPRT deficiency is determined by using a selective medium containing base analogues 8-azaguanine (8 AZ).

Endpoints: Gene mutation. Qualitative: Formation of cell colonies in a selective medium. Quantitative: Induced mutation frequencies are based on cell survival and cloning efficiency.

Strengths: Mammalian organization of genetic material; Forward mutation assay; Fast generation time; Stable karyotype; Easy to culture; May be particularly well-suited to quantitation as HGPRT locus mutants may not show replicative advantages/disadvantages over wild-type cells under nonselective conditions.

Weaknesses: Mutation measured at a single locus; Requires metabolic activation; Requires additional validation; Lacks pharmacological relevance; Fairly high spontaneous mutation rate (1^{-5} x 10^{-6}); Long optimal expression period (7 days).

Status of Development: Being implemented.

Describe: Needs additional validation.

Applications: Multimedia.

Samples: Pure Chemicals: N-Nitroso compounds, Alkylating agents, Primarily organics mixtures. Complex Mixtures: Ambient; Industrial; Energy Related.

Duration: 4 weeks.

Cost: $3,000.

Interpretation: Growth of the treated HGPRT competent cells in a selective medium suggests genetic change.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemical Branch; HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); N.E. Kowal, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7477).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, A. Mitchell; Gulf South Research Institute, P.O. Box 26518, New Orleans, LA 70186, N. Gruener.

Program Office Support: OHEE.

References: 1) Krahn, D.F., and C. Heidelburger. Proc. Natl. Acad. Sci., 73:188-192, 1977. 2) Artlett, et al. Mutation Res., 33: 261-278, 1975.

12110 DROSOPHILA MELANOGASTER, SEX LINKED RECESSIVE LETHAL

Biological Activity Detected: Mutagenicity.

Principle: Wild type males are treated with the test chemical and mated with untreated females with a marked chromosome. F_1 females are sib-mated and the progeny are scored for the presence of x-linked recessive lethals.

Endpoints: Qualitative: Change in sex ratios in F_2 generation. Quantitative: Point mutations and small delections may be scored in germ cells.

Strengths: Higher organism, genetically well characterized; Multiple loci available for detection of genetic alterations; Some metabolic processes similar to that of mammals; Small number of large chromosomes; Broad spectrum of genetic events can be detected and scored; Wealth of mutant strains makes possible detailed analysis of induced genetic changes.

Weaknesses: Short life span makes organism unsuitable for chronic exposure studies; Limited use for testing pesticides due to extreme toxicity.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents, Nitrosamines, Halogenated ethers. Complex Mixtures: Protocols not yet developed.

Duration: 4 to 6 weeks.

Cost: $4,000 to $6,500.

Interpretation: Change in the sex ratio in the F_2 progeny of the test population indicates mutation.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu; U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, A. Mitchell.

Program Office Support: OHEE.

References: 1) Vogel, E., and F.H. Sobels. The Function of *Drosophila* in Genetic Toxicology Testing. In: Chemical Mutagens: Principles and Methods for Their Detection. Vol. 4. A. Hollaender, ed. Plenum Press, NY, 1976. pp. 93-132. 2) Sobels, F.H., and E. Vogel. The Capacity of *Drosophila* for Detecting Relevant Genetic Damage. Mutation Res., 41:95-106, 1976. 3) Legator, M.S., and S. Zimmering. Gen. Toxicol. Ann. Rev. Pharmacol., 387-408, 1975.

12111 TRADESCANTIA STAMEN HAIR

Biological Activity Detected: Mutagenicity.

Principle: Mutation in petals and stamen hair in clones heterozygous for flower color is detected as a change in pigmentation.

Endpoints: Qualitative: Change in stamen hair cells' color from blue to pink. Quantitative: Mutational events per stamen hair and dose-response relationship can be established.

Strengths: Can detect broad spectrum of genetic events; Can be used to monitor in situ environment; Can detect mutagens in the gaseous phase; Eukaryotic organization of genetic material; Many mutational events can be observed directly; System appears highly sensitive to physical and chemical mutagens.

Weaknesses: Lacks pharmacological relevance; May not be suitable for evaluating many compounds requiring mammalian metabolic activation.

Status of Development: Developmental.

Describe: The *Tradescantia* system was initially developed (and is well-suited) for study of radiation effects. The system is applicable to at least some chemical mutagens and is currently under development for that purpose.

Applications: Air; Water.

Samples: Pure Chemicals: Organics, Nitroso derivatives, Polyclichydrocarbons, Nucleic acid bases, and analogs, Hydroxylamines, Hydrasine. Complex Mixtures: Ambient - drinking water, air; Industrial - soil.

Duration: 2 to 5 weeks.

Cost: $500 to $700, depending upon the test protocol.

Interpretation: The change in stamen hair cells' color from blue to pink suggests mutagenicity.

Level of Complexity: I.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section; HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); L.J. McCabe, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7211).

Grant/Contract Laboratory Involved and Principal Investigators: Brookhaven National Laboratories, Long Island, New York, L. Shirer; U. of Missouri, Columbia, MO 65201, J.T. O'Connor; Stanford Research Institute, Menlo Park, CA 94205, G. Newell.

Program Office Support: OHEE; OPP.

References: 1) Sparrow, A.H., et al. Mutation Res., 26:265-276, 1974. 2) Underbrink, A.G., et al. In: Chemical Mutagens: Principles and Methods for Their Detection. Vol. 3. A. Hollaender, ed. Plenum Press, NY, 1973. pp. 171-207. 3) McNulty, P.J., et al. Mutation Res., 44:235-246, 1977.

12112 MAIZE WAXY LOCUS ASSAY

Biological Activity Detected: Mutagenicity.

Principle: The assay is based on the change from the ability of the plants to synthesize amylose to the inability to synthesize this compound. Pollen from the treated plants is stained with iodine. Mutated pollen grains are stained purple.

Endpoints: Qualitative: Change in pollen grain color from yellow to purple. Quantitative: Induced mutation frequency is expressed.

Strengths: Test is performed in-vivo representing relevant conditions of exposure; Damage to germ cells is measured.

Weaknesses: Time consuming.

Status of Development: Developmental.

Describe: Few pure compounds or mixtures have been tested in this system. This test system appears promising but needs validation.

Applications: Air; Soil; Multimedia.

Samples: Pure Chemicals: Pesticides. Complex Mixtures: Industrial.

Duration: 8 weeks.

Cost: Unknown.

Interpretation: Change in pollen grain color after staining suggests mutation.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: U. of Illinois, Urbana, IL 61801, M. Plewa.

Program Office Support: OPP.

References: 1) Plewa, M., and J. Gentile. Maize Cooper. Newsletter, 50:44, 1976. 2) Plewa, M., and J. Gentile. Mutation Res., 38:287-292, 1976.

12113 IN-VIVO CYTOGENETICS IN MICE

Biological Activity Detected: Mutagenicity.

Principle: Chemicals are administered into mice through various routes. After a specific period, treated animals are sacrificed. Bone marrow and spermatogonial cells are analyzed for chromosomal aberrations.

Endpoints: Qualitative: Observation of chromosomal and chromatid breaks. Quantitative: Number of breaks/cell as compared to the control, provides a quantitative assessment of treatment response.

Strengths: In-vivo bioassay provides the benefit of intact pharmokinetics.

Weaknesses: Expensive; Requires a well trained personnel to perform the test and interpret the data.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents. Complex Mixtures: Protocol not yet developed.

Duration: 3 weeks.

Cost: $3,000 to $6,500/chemical.

Interpretation: A significant increase in chromosomal aberrations over the control suggests mutation.

Level of Complexity: 5.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section; HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); L. Claxton, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2942); J.F. Stara, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7407).

Grant/Contract Laboratory Involved and Principal Investigators: U. of Texas, Medical Branch, Galveston, TX 77550, M. Legator; Stanford Research Institute, Menlo Park, CA 94205, G. Newell.

Program Office Support: OHEE, OPP.

References: 1) Evans, H.J. In: Chemical Mutagens: Principles and Methods for Their Detection. Vol. 4. A. Hollander, ed. Plenum Press, NY, 1977. 2) Schmid, W. In: Chemical Mutagens: Principles and Methods for Their Detection. Vol. 4. A. Hollander, ed. Plenum Press, NY, 1977. pp. 31-52.

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1221 BACILLUS SUBTILIS REC$^-$

Biological Activity Detected: Toxicity; Primary DNA damage.

Principle: The DNA recombinational repair deficient and proficient strains are streaked out along the intersecting lines. The test chemical is spotted at the intersection. The differential killing between the repair deficient and proficient strains is used as a criteria of DNA damage.

Endpoints: Qualitative: A comparison is made between the zone of growth inhibition for the repair deficient and proficient strains. Quantitative: N/A.

Strengths: Very rapid and versatile bioassay; Inexpensive.

Weaknesses: Requires fairly large amount of test substances for testing; Not suitable for substances which do not diffuse readily in agar.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents, Nitroso compounds, Polynuclear aromatics, Nitroso derivatives; Pesticides. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other.

Duration: 2 to 3 weeks.

Cost: $200.

Interpretation: Relative sizes of killing zones in repair-proficient and repair-deficient stains indicate primary damage to DNA.

Level of Complexity: 1.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); L. Claxton, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2942); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); J.L. Huisingh, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2948).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, V.F. Simmons.

Program Office Support: OHEE; OPP.

References: 1) Kada, T., K. Tutikawa, and Y. Sadaie. Mutation Res., 16:165-174, 1972.

1222 ESCHERICHIA COLI, Pol A^-.

Biological Activity Detected: Toxicity; Primary DNA damage.

Principle: The assay measures the differential killing between DNA repair proficient *Escherichia coli* strain (W3110, Pol A^+) and DNA repair deficient strain (P3478, Pol A^-) as affected by environmental toxicants.

Endpoints: *Qualitative*: Differential killing between DNA repair proficient and deficient strain after treatment with a test-substance. *Quantitative*: Liquid suspension test provides a quantitative measure of primary DNA damage.

Strengths: The assay is well suited for detecting chemicals causing frame shift mutations; Adequate data base is present on this bioassay; Genetically well-characterized system; Rapid; Inexpensive; Well validated as a test for gene mutation; Works well with in-vitro metabolizing microsome fractions; Can be used as indicator organism in host-mediated assays.

Weaknesses: Reverse mutation assay requiring several strains to permit detection of a broad spectrum of compounds; Requires metabolic activation; Lacks pharmacological relevance, Prokaryotic organization of genetic material.

Status of Development: Validated.

Describe: N/A.

Applications: Multimedia.

Samples: *Pure Chemicals*: Alkylating agents, Nitroso compounds, Polynuclear aromatics, Nitroso derivatives, pesticides. *Complex Mixtures*: Ambient; Industrial; Energy Related; Transportation Related; Other.

Duration: 2 to 4 weeks.

Cost: $500.

Interpretation: Relative sizes of killing zones in repair-proficient and repair-deficient stains indicate primary damage to DNA.

Level of Complexity: I.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); L. Claxton, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2942).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, V.F. Simmons.

Program Office Support: OHEE; OPP.

References: 1) Rosenbranz, H.S., et al. Mutation Res., 41:61-70, 1976. 2) Rosenbranz, H.S. Ann. Res. Microbiol., 27:383-401, 1973. 3) Rosenbranz, H.S. Cancer Res., 33:458-459, 1973.

1223 MITOTIC RECOMBINATION AND GENE CONVERSION IN SACCHAROMYCES CEREVISIAE

Biological Activity Detected: Primary DNA damage.

Principle: Recombination of reciprocal type, mitotic recombination, and the non-reciprocal type mitotic gene conversion are used for assessing the DNA damaging potential of environmental chemicals.

Endpoints: Qualitative: Appearance of twin spots and growth in selective medium. Quantitative: The degree of mitotic crossing-over is evaluated by the frequency of twin spot sectors and that of mitotic gene conversion by the differential growth in a selective medium.

Strengths: The diploid cells with eukaryotic chromosomal organization; Rapid; Inexpensive.

Weaknesses: Less versatile due to problems associated with cell wall permeability and with coupling of metabolic activation; Inadequate data base showing the reliability of this assay system.

Status of Development: Being implemented.

Describe: This test system needs to be validated with a variety of classes of compounds. Further work is needed in understanding the mechanism and significance of mitotic recombination and crossing-over.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents, Nitrosamines, Carbamates, Nucleic acid bases and analogs, Aromatic amines. Complex Mixtures: Protocol not yet developed.

Duration: 2 to 3 weeks.

Cost: $200 to $500.

Interpretation: The appearance of twin spots and growth in a selective medium suggests mutation.

Level of Complexity: [illegible]

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, V.F. Simmons.

Program Office Support: OHEE; OPP.

References: 1) Zimmermann, F.K. Mutation Res., 31:71-86, 1975. 2) Brusick, D.J., and V.W. Mayer. Envir. Hlth. Prospect., 6:83, 1973.

1224 UNSCHEDULED DNA SYNTHESIS (UDS)

Biological Activity Detected: Primary DNA.

Principle: This assay evaluates the test compounds for their ability to induce unscheduled DNA synthesis (UDS) in human diploid WI38 fibroblasts blocked in the G_1 phase.

Endpoints: Qualitative: Incorporation of ^3H thymidine. Quantitative: dpm ^3H thymidine per μg or μmole of DNA.

Strengths: DNA repair can be measured in human cells in culture; Similar studies can be performed in animals using peripheral leucocytes; The latter permit comparison between in-vitro and in-vivo exposures to carcinogens or mutagens.

Weaknesses: The precise type of molecular binding between carcinogens and DNA which triggers excision repair is unknown; DNA repair synthesis does not measure residual damage to DNA.

Status of Development: Validated.

Describe: The mechanism of UDS is still not completely understood.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylalois; Alkylating agents, Nitroso compounds, Polynuclear aromatics. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other.

Duration: 4 to 6 weeks.

Cost: $350 to $2,000.

Interpretation: The incorporation of labeled nucleotide precursors into the cells arrested in the G_1 phase after treatment is used as a criteria of the ability of the test material to cause primary DNA damage.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, A. Mitchell.

Program Office Support: OHEE; OPP.

References: 1) Stich, H., and S. Laighes. DNA Repair and Chemical Carcinogenesis. Pathobiol. Ann., 3:342-376, 1973. 2) San, R.H., and H.F. Stich. DNA Repair Synthesis of Cultured Human Cells as a Rapid Bioassay for Chemical Carcinogens. Int. J. Cancer, 16: 284-291, 1975. 3) Williams, G.M. Detection of Chemical Carcinogens by Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures. Cancer Res., 37:1845-1851, 1977. 4) Simmon, V.F., A.D. Mitchell, and T.A. Jorgenson. Evaluation of Selected Pesticides as Chemical Mutagens: In-vitro and In-vivo Studies. Ann. Rep., Envir. Hlth. Effects Series, EPA-600/1-77-028, 1977.

1225 SISTER-CHROMATID EXCHANGE FORMATION (SCE)

Biological Activity Detected: Primary DNA damage.

Principle: SCE involves a reciprocal exchange between sister-chromatids which does not result in a change in the overall chromosonal morphology. SCE may be observed as darkly staining and lightly staining chromatids after growth in BUdR for two successive cell generations and subsequent-staining with fluorochrome dyes.

Endpoints: Qualitative: Observation of sister-chromatid exchanges of metaphase. Quantitative: SCE/cell are expressed.

Strengths: Rapid; Relatively economical; Very sensitive; Can be tested in-vivo or in-vitro.

Weaknesses: Mechanism and significance of SCE is not understood; No clear relationship between SCE and chromosomal breaks has been established.

Status of Development: Being implemented.

Describe: The validation of this system is near completion in several laboratories.

Applications: Multimedia.

Samples: Pure Chemicals: Alkylating agents, Mycotoxins, Halogenated hydrocarbons, Ureas and Thioureas, Nitro derivatives.

Complex Mixtures: Protocol not yet developed.

Duration: 2 to 4 weeks.

Cost: $1,000 to $1,200.

Interpretation: Test agents inducing signigicant numbers of sister-chromatid exchanges may be regarded as potential DNA-damaging agents for animals and man.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch; ERL-NAR, Toxicology Branch, Genetic Toxicology Team.

Persons to Contact: M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); S.S. Sandhu, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); A.R. Malcolm, U.S. EPA, ERL-NAR, South Ferry Rd., Narragansett, RI 02882, (FTS 838-4843); G.G. Pesch, U.S. EPA, ERL-NAR, South Ferry Rd., Narragansett, RI 02882, (FTS 838-4843).

Grant/Contract Laboratory Involved and Principal Investigators: Stanford Research Institute, Menlo Park, CA 94205, V.F. Simmons.

Program Office Support: OHEE; OPP.

References: 1) Perry, P., and H.J. Evans. Nature, 258;121-125, 1975. 2) Latt, S.A. Proc. Natl. Acad. Sci., 70:3395-3399, 1973. 3) Popescur, N.C., et al. F. Natl. Cancer Inst., 59:289-293, 1977.

1226 IN-VIVO ASSESSMENT OF DNA DAMAGE

Biological Activity Detected: Primary DNA damage.

Principle: Detection of in-vivo DNA repair activity that is stimulated by chemical carcinogens.

Endpoints: Qualitative: Initial endpoint-measurement of DNA molecular weight change. Quantitative: Molecular weight distribution of cleaved DNA strands.

Strengths: Detects DNA damage in-vivo; Assay is done on biopsy material; Non-destructive, animal sampled can later be scored for tumors to validate assay.

Weaknesses: Not yet apparent; Mainly technical.

Status of Development: Developmental.

Describe: Still in early stages of development.

Applications: Multimedia.

Samples: Pure Chemicals: Organic compounds, Inorganics, Heavy metals. Complex Mixtures: Ambient - water; Industrial; Energy Related; Transportation Related; Other.

Duration: Variable, but not to exceed 3 months once validated.

Cost: Not yet determined.

Interpretation: Mutagenic and carcinogenic agents act through damage of DNA. This method will quantitate DNA damage.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213).

Grant/Contract Laboratory Involved and Principal Investigators: Ohio State University, Chemical Biomedical Environmental Research Group, Columbus, OH 43210, R.W. Hart, (FTS 940-9375).

Program Office Support: OHEE; OWHM.

References: 1) Brash, et al. N.Y. Acad. J.C., 1977. 2) Brash and Hart, R.W. Envir. Hlth. Perspect., 1978. In press.

1227 INTACT RODENT HEPATOCYTES IN PRIMARY CULTURE

Biological Activity Detected: Toxicity; Presumptive mutagenicity; Oncogenicity; DNA damage.

Principle: Detects interaction of chemical agents which result in DNA damage. This interaction is detected as unscheduled DNA synthesis using radiotracer, centrifugal and autoradiographic techniques.

Endpoints: Qualitative: A positive response suggests potential to act as a mutagen or carcinogen. Quantitative: Can quantify the number of grains/nucleus which increases with increased dose.

Strengths: Rapid; Economical; The entire genome is the target; Maintains several functions of tissue of origin, thereby activating many different chemicals; Not limited to direct acting compounds.

Weaknesses: Represents only a single target organ; System lacks validation; May not detect promotors or co-carcinogens.

Status of Development: Developmental.

Describe: This system is now in the process of validation in several laboratories. It has not yet been applied to unknown compounds.

Applications: Multimedia.

Samples: Pure Chemicals: Aromatic amines, Polycyclics, Alkylators, Hormones. Complex Mixtures: Not yet determined.

Duration: 2 days to 2 weeks, depending on technique.

Cost: $500 to $2,000.

Interpretation: This test is considered a reliable screen for prioritization of chemical testing in more complex in-vivo systems. It suggests that the chemical reaches the genome and requires further analysis.

Level of Complexity: 3 to 4.

OHEE Laboratory Involved: National Center for Toxicological Research, Division of Mutagenesis Research, Somatic Cell Section; HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Cellular Biology Section.

Persons to Contact: D.A. Casciano, National Center for Toxicological Research, Jefferson, AR 72079, (FTS 740-4573); J.L. Huisingh, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2948).

Grant/Contract Laboratory Involved and Principal Investigators: American Health Foundation, Naylor Dana Institute for Disease Prevention, Hammond House Road, Valhalla, NY 10595, G.M. William.

Program Office Support: OHEE.

References: 1) Kitagawa, T., et al. Cancer Res., 35:3682-3692, 1975. 2) Michalopoulos, G., et al. Life Sciences, 18:1139-1144, 1976. 3) Williams, G.M. Cancer Letters, 1:231-236, 1976. 4) San, R.H.C., and H.F. Stich. Intl. J. Cancer, 7:65-74, 1971.

1228 IN-VIVO DNA BINDING

Biological Activity Detected: Binding of chemical to DNA.

Principle: This test system attempts to correlate binding with DNA repair and tumorigenicity. Various rodent stains will be treated with the chemical carcinogen DNA from presumptive target tissue analyzed for DNA-carcinogen adducts by radiometric and/or fluorometric techniques, measuring the rate of excision of the bound chemical.

Endpoints: Qualitative: Adducts will be determined by chromatographic procedures. Quantitative: Can quantify amount of carcinogen bound/unit DNA (μ mole carcinogen/mole DNA-phosphate).

Strengths: A direct measurement of the extent of carcinogen interaction with DNA; Allows measurement of total DNA damage.

Weaknesses: A considerable portion of the DNA-carcinogen adducts may have little or no biological relevance.

Status of Development: Developmental.

Describe: Initial experiments are being carried out to provide background for development of in-vitro DNA repair assay.

Applications: Multimedia.

Samples: Pure Chemicals: Organic-metallic compounds, Organics. Complex Mixtures: N/A.

Duration: 2 weeks.

Cost: Not yet determined.

Interpretation: This test measures the direct interaction of the test compound with genetic material as an indication of carcinogenic and mutagenic activity.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

Persons to Contact: B. Daniel, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7482).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OWHM.

References: Not yet available.

1229 CHINESE HAMSTER CELLS (CHO) UNSCHEDULED DNA SYNTHESIS (UDS)

Biological Activity Detected: DNA Repair.
Principle: Repair of induced damage to DNA is detected as unscheduled DNA synthesis via incorporation of ^{3}H thymidine.
Endpoints: Qualitative: Unscheduled DNA synthesis is measured and compared to controls. Quantitative: Amount of unscheduled DNA synthesis per cell may be determined.
Strengths: Rapid; Relatively low cost; DNA repair is probably a more sensitive detector of DNA damage than are chromosomal aberrations.
Weaknesses: The in-vitro cell assay lacks pharmacological relevance; Requires metabolic activation.
Status of Development: Being implemented.
Describe: The test is presently being applied to standard compounds and some selected unknowns. It is still being refined and modified.
Applications: Water; Multimedia.
Samples: Pure Chemicals: Organics (EMS), Heavy metals, UV radiation. Complex Mixtures: Energy Related - JP-5 jet fuel.
Duration: 2 to 3 weeks.
Cost: $1,000 to $1,200/compound.
Interpretation: Agents inducing significant unscheduled DNA synthesis represent potential carcinogens/mutagens for animals and man.
Level of Complexity: 2.
OHEE Laboratory Involved: ERL-NAR, Toxicology Branch, Genetic Toxicology Team.
Persons to Contact: E. Jackim, U.S. EPA, ERL-NAR, South Ferry Rd., Narragansett, RI 02882, (FTS 838-4843 X229, X310); A.R. Malcolm, U.S. EPA, ERL-NAR, South Ferry Rd., Narragansett, RI 02882, (FTS 838-4843 X238, X247).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OHEE.
References: San, R.H.C., and H.F. Stitch. Int. J. Cancer, 16:284-291, 1975. 2) Martin, C.N., et al. Cancer Letters, 2:355-360, 1977. 3) Trosko, J.E., and J.D. Yager. Exp. Cell Res., 88:47-55, 1974.

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1231 C3H10T1/2CL8 MOUSE EMBRYO FIBROBLAST ONCOGENIC TRANSFORMATION WITH EXOGENOUS METABOLIC ACTIVATION

Biological Activity Detected: Presumptive oncogenicity.

Principle: Normal cells in log phase are treated with the test agent. Four weeks after the cells have attained confluence they are scored for morphologically transformed foci (clones of cells). These transformed cells will give rise to tumors when injected into immunosupressed syngeneic animals.

Endpoints: Qualitative: Appearance of morphologically transformed foci. Quantitative: Simultaneous cytotoxicity experiments are performed to obtain the lethal toxicity of the agent and transformation is then adjusted for that toxicity.

Strengths: This system is easy to score; Has an extremely low background of spontaneous transformation; Is particularly sensitive to PAH and their derivatives; Has metabolic activation capability; Can be used to detect tumor promotors and initiators.

Weaknesses: These mouse embryo cells are aneuploid; Requires 6 weeks to complete the experiment; Seems to be somewhat refractory to the carcinogenic effects of alkylating agents, aflatoxin B_1 and some aromatic amines.

Status of Development: Developmental.

Describe: The C3H10T1/2 system is being modified by the addition of exogenous metabolic activation capability, giving, therefore, increased sensitivity towards a broad range of chemical carcinogens.

Applications: Air.

Samples: Pure Chemicals: Polycyclic aromatic hydrocarbons.
Complex Mixtures: N/A.

Duration: 5 to 6 weeks.

Cost/sample or chemical: $5,000 to $7,000.

Interpretation: The appearance of morphologically altered clones of cells indicates oncogenic transformation.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Metabolic Effects Section.

Persons to Contact: S. Nesnow, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: Microbiological Associates, Bethesda, MD 20014, R. Kouri, L. Schectman.

Program Office Support: OEMI.

References: 1) Reznikoff, et al. Cancer Res., 33:3231-3249, 1973. 2) Nesnow, et al. Cancer Res., 36:1801-1808, 1976. 3) Mondal, et al. Cancer Res., 36:2254-2260, 1976. 4) Bertram. Cancer Res., 37:514-523, 1977. 5) Benedict, et al. Cancer Res., 37:2202-2208, 1977.

1232 C3H10T1/2CL8 MOUSE EMBRYO FIBROBLAST ONCOGENIC TRANSFORMATION

Biological Activity Detected: Presumptive oncogenicity.

Principle: Normal cells in log phase are treated with the test agent. Four weeks after the cells have attained confluence they are scored for morphologically transformed foci (clones of cells). These transformed cells will give rise to tumors when injected into immunosupressed syngeneic animals.

Endpoints: Qualitative: Appearance of morphologically transformed foci. Quantitative: Simultaneous cytotoxicity experiments are performed to obtain the lethal toxicity of the agent and the transformation is then adjusted for that toxicity.

Strengths: This system is easy to score; Has an extremely low background of spontaneous transformation; Is particularly sensitive to PAH and their derivatives; Has metabolic activation capability; Can be used to detect tumor promotors and initiators.

Weaknesses: These mouse embryo cells are aneuploid; Requires 6 weeks to complete the experiment; Seems to be somewhat refractory to the carcinogenic effects of alkylating agents, aflatoxin B$_1$, and some aromatic amines.

Status of Development: Being implemented.

Describe: The C3H10T1/2 system is being modified so that its metabolic activation capability is increased, giving, therefore, increased sensitivity towards a broad range of chemical carcinogens.

Applications: Multimedia.

Samples: Pure Chemicals: Polycyclic aromatic hydrocarbons, Aromatic azo dyes, aromatic amines, pesticides. Complex Mixtures: Model particulates.

Duration: 5 to 6 weeks.

Cost/sample: $5,700 to $7,000.

Interpretation: The appearance of morphologically altered clones indicates oncogenic transformation.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Metabolic Effects Section.

Persons to Contact: S. Nesnow, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: In-house.

Program Office Support: OHEE; OPP.

References: 1) Reznikoff, et al. Cancer Res., 33:3231-3249, 1973. 2) Nesnow, et al. Cancer Res., 36:1801-1808, 1976. 3) Mondal, et al. Cancer Res., 36:2254-2260, 1976. 4) Bertram. Cancer Res., 37:514-523, 1977. 5) Benedict, et al. Cancer Res., 37:2202-2208, 1977.

1233 BHK-21 MAMMALIAN CELL ONCOGENIC TRANSFORMATION

Biological Activity Detected: Presumptive oncogenicity.

Principle: Normal baby hamster kidney fibroblasts do not grow in soft agar. After treatment with carcinogens these cells do grow in soft agar and will give tumors when injected into syngeneic animals.

Endpoints: Qualitative: Cells which grow in soft agar are considered transformed. Quantitative: Colony formation is scored.

Strengths: Not yet known.

Weaknesses: Not yet known.

Status of Development: Developmental.

Describe: Testing of unconcentrated and concentrated wastewater samples.

Applications: Water.

Samples: Pure Chemicals: PAH. Complex Mixtures: Wastewaters.

Duration: 3 weeks.

Cost/sample or chemical: Not yet known.

Interpretation: A positive result suggests a possible carcinogenic material.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Field Studies Division, Toxicological Assessment Branch.

Persons to Contact: H. Pahren, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7217).

Grant/Contract Laboratory Involved and Principal Investigators: Syracuse Research Corporation, Merrill Lane, Syracuse, NY 13210, J. Saxena.

Program Office Support: OHEE.

References: 1) Bouck, N., and G. diMayorca. Nature, 264:722-727, 1976.

1234 SYRIAN HAMSTER EMBRYO ONCOGENIC TRANSFORMATION (FOCUS ASSAY)

Biological Activity Detected: Presumptive oncogenicity.

Principle: Freshly isolated cells from hamster fetuses are seeded into dishes, passaged twice, and then treated for two consecutive rhree drug treatments with the test agents. Toxicity is scored 1 to 2 days after the experiment is begun and the transformation (appearance of morphologically transformed foci) is scored 10 days after toxicity.

Endpoints: Qualitative: Appearance of morphologically transformed foci. Quantitative: Simultaneous cytotoxicity experiments are performed to obtain the lethal toxicity of the agent and the transformation is then adjusted for that toxicity.

Strengths: These cells have high metabolic activation capability; Are diploid; Respond to a wide variety of different chemical agents; Few false positives are known.

Weaknesses: Variability within the assay due to variations in obtaining and preparing viable primary cell cultures; Observable spontaneous transformation background; Difficulty in scoring.

Status of Development: Being implemented.

Describe: The syrian hamster embryo biassay is being evaluated for use in the evaluation of particulate samples.

Applications: Air.

Samples: Pure Chemicals: Polycyclic aromatic hydrocarbons. Complex Mixtures: Model particulates.

Duration: 1 month.

Cost/assay: $4,000 to $5,000.

Interpretation: The appearance of morphologically altered clones of cells indicates oncogenic transformation.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP; Environmental Toxicology Division, Biochemistry Branch, Metabolic Effects Section.

Persons to Contact: S. Nesnow, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693); M.D. Waters, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: In-house.

Program Office Support: OHEE.

References: 1) Casto, B.C., N. Janosko, and J.A. DiPaolo. Cancer Res., 37:3508-3515, 1977.

1235 BALB 3T3 ONCOGENIC TRANSFORMATION AND MUTAGENESIS WITH EXOGENOUS METABOLIC ACTIVATION

Biological Activity Detected: Mutagenicity; Presumptive oncogenicity.

Principle: Mammalian cell clones of BALB 3T3 clone A13 undergo malignant transformation upon treatment with known carcinogens. Extent of transformation is expressed in focus formation and altered morphology, increased saturation density and enhanced plating efficiency in soft agar. Simultaneously these cells also undergo a permanent genetic change at the ouabain locus. Tumor induction is observed in recipient animals by reinjecting transformed cells.

Endpoints: Qualitative: Appearance of morphological transformed foci for transformation and clones of cells which grow in ouabain supplanted media. Quantitative: Number of observed foci adjusted for the cytotoxic effect of the agent.

Strengths: BALB 3T3 cells are mouse embryo fibroblasts which have the capability to activate 3-methylcholanthrene to metabolites which transform these cells; Direct acting alkylating agents such as MNNG are also effective transforming agents.

Weaknesses: These cells are not transformed by the carcinogens benzo!a!pyrene or 6-aminochrysene and possibly other potent carcinogens unless exogeneous metabolic activation is provided.

Status of Development: Developmental.

Describe: The addition of rat liver preprarations to activate carcinogens and mutagens to make this assay system more sensitive is currently underway.

Applications: Air.

Samples: Pure Chemicals: PAH, Aromatic amines. Complex Mixtures: Transportation Related.

Duration: 5 to 6 weeks.

Cost/sample or chemical: $5,000 to $7,000

Interpretation: Morphologically altered clones of cells indicate oncogenic transformation. Cells growing in the presence of ouabain are indicative of a mutagenic change.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Biochemistry Branch, Metabolic Effects Section.

Persons to Contact: S. Nesnow, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2693).

Grant/Contract Laboratory Involved and Principal Investigators: Microbiological Associates, Bethesda, MD 20014, L. Schectman, R. Kouri.

Program Office Support: OEMI.

References: 1) Kakunaga, T. A Quantitative Assay for Malignant Transformation by Chemical Carcinogens Using Clone from BALB 3T3. Int. J. Cancer, 12:463, 1973.

1236 BALB 3T3 ONCOGENIC TRANSFORMATION

Biological Activity Detected: Presumptive oncogenicity.

Principle: Mammalian cell clones of BALB 3T3 clone A13 undergo malignant transformation with known carcinogens. Extent of transformation is expressed in focus formation and altered morphology, increased saturation density, and enhanced plating efficiency in soft agar. Tumor induction is observed in recipient animals by reinjecting transformed cells.

Endpoints: Qualitative: Appearance of morphological transformed foci. Quantitative: Number of observed foci adjusted for the cytotoxic effect of the agent.

Strengths: BALB 3T3 cells are mouse embryo fibroblasts which have the capability to activate 3-methylcholanthrene to metabolites which transform these cells; Direct acting alkylating agents such as MNNG are also effective transforming agents.

Weaknesses: These cells are not transformed by the carcinogens benzo(a)pyrene or 6-aminochrysene and possibly other potent carcinogens.

Status of Development: Being implemented.

Describe: Currently BALB/T3 cell culture is used for routine testing, but other cell lines (e.g. epithelial) are being investigated in an effort to increase sensitivity.

Applications: Water.

Samples: Pure Chemicals: Organics. Complex Mixtures: Ambient - drinking water; Other - advanced waste treatment, concentrate effluent.

Duration: 5 to 6 weeks.

Cost/sample or chemical: $5,000 to $7,000.

Interpretation: A positive result indicates possible carcinogenesis.

Level of Complexity: 2.

OHEE Laboratory Involved: HERL-CIN, Field Studies Division, Toxicological Assessment Branch; HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

Persons to Contact: J.P. Bercz, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7432); N.E. Kowal, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7477); R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213).

Grant/Contract Laboratory Involved and Principal Investigators: U. of Cincinnati, Medical Center, Cincinnati, OH 45221, J.C. Loper and D. Lang; Gulf South Research Institute, P.O. Box 26518, New Orleans, LA 70186, N. Gruener.

Program Office Support: OHEE.

References: 1) Kakunaga, T. A Quantitative Assay for Malignant Transformation by Chemical Carcinogens Using Clone From BALB 3T3. Int. J. Cancer, 12:463, 1973.

1237 PULMONARY ADENOMA BIOASSAY IN MICE

Biological Activity Detected: Presumptive oncogenicity.

Principle: After 13 weeks no untreated strain A mice develop lung tumors; however, after 1 year 100% develop lung tumors. When treated with a carcinogen, lung tumors start developing within 13 weeks.

Endpoints: Qualitative: Tumor formation. Quantitative: Average number of tumors/mouse lung in the treated animals as compared to the positive controls (urethane) and untreated controls.

Strengths: Relatively rapid in-vivo carcinogenesis bioassay.

Weaknesses: The adenoma (pulmonary) has no counterpart in human neoplastic pathology.

Status of Development: Being implemented.

Describe: The system has been developed and used in other laboratories, see references.

Applications: Multimedia.

Samples: Pure Chemicals: Metalic compounds, Food additives, chemotherapeutic agents. Complex Mixtures: Industrial; Transportation Related - diesel particulate.

Duration: 13 to 30 weeks.

Cost/sample or chemical: Not yet known.

Interpretation: The formation of visually observable lung nodules indicates oncogenicity of the test substance.

Level of Complexity: 4.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Functional Pathology Branch.

Persons to Contact: J. Orthoefer, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7434).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Shimkin and Stoner. Lung Tumors in Mice: Application to Carcinogenesis Bioassay. Adv. in Cancer Res., 21:1-58, 1975. 2) Stoner, et al. Test for Carcinogenicity of Metallic Compounds by the Pulmonary Tumor Response in Strain A Mice. Cancer Res., 36:1744-1747, May 1976.

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1241 TUMOR INDUCTION IN MASSIVE CRUSTACEANS, MOLLUSCS, AND TELIOST FISH

Biological Activity Detected: Presumptive oncogenicity.

Principle: Animals are exposed to known carcinogens under laboratory conditions and histopathology is performed. Also, feral animals are surveyed for histopathological abnormalities and correlations are established with tissue residues and water concentrations.

Endpoints: Qualitative: Not supplied. Quantitative: Correlation of exposure/response.

Strengths: May have value as sentinel system for water quality and as a model system.

Weaknesses: Field correlation requires laboratory validation.

Status of Development: Being implemented.

Describe: Not supplied.

Applications: Water.

Samples: Pure Chemicals: PNA's. Complex Mixtures: Ambient - water; Industrial - water.

Duration: Not supplied.

Cost: Not supplied.

Interpretation: Not supplied.

Level of Complexity: Not supplied.

OHEE Laboratory Involved: ERL-GB.

Persons to Contact: J. Couch, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).

Grant/Contract Laboratory Involved and Principal Investigators: U. of Oregon, Eugene, OR 97403, M. Mix; U. of Southern Mississippi, Hattiesburg, MI 39401, B.J. Martin.

Program Office Support: OEMI; OHEE.

References: Not supplied.

1242 MFO INDUCTION AS AN INDICATOR OF TOXICITY EXPOSURE

Biological Activity Detected: Toxicity; Mutagenicity; Presumptive oncogenicity.
Principle: Fetal and laboratory reared animals are exposed to ambient environmental conditions as sentinal organisms.
Endpoints: Qualitative: Not supplied. Quantitative: Degree of induction of MFO.
Strengths: Pre-pre screen method for water quality; Provides guidance for chemical analysis programs.
Weaknesses: Nonspecific.
Status of Development: Developmental.
Describe: Not supplied.
Applications: Water.
Samples: Pure Chemicals: PNA's; Complex Mixtures: Ambient - estuarine/marine.
Duration: Not supplied.
Cost: Not supplied.
Interpretation: Positive test indicates that the test animal has recently been exposed to inducer(s) of MFO systems.
Level of Complexity: Not supplied.
OHEE Laboratory Involved: ERL-GB.
Persons to Contact: N. Richards, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011); P. Schoor, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).
Grant/Contract Laboratory Involved and Principal Investigators: U. of West Florida, Pensacola, FL 32504, R. Rao.
Program Office Support: OHEE.
References: Not supplied.

1243 LIMB REGENERATION SYSTEM

Biological Activity Detected: Differentiation/teratology.
Principle: Crustacean limbs are removed at predetermined breakpoints and regenerated by a precise sequence of biochemical events.
Endpoints: Qualitative: Interference with limb regeneration - determined by gross morphology and histopathology. Quantitative: Not supplied.
Strengths: Appropriate for marine samples.
Weaknesses: Difficult to extrapolate to humans.
Status of Development: Being implemented.
Describe: Not supplied.
Applications: Water.
Samples: Pure Chemicals: PCP, Colchicine, PNA's. Complex Mixtures: Ambient - estuarine/marine; Energy Related - drilling fluids.
Duration: Not supplied.
Cost: Not supplied.
Interpretation: Pre-screen for teratogens.
Level of Complexity: Not supplied.
OHEE Laboratory Involved: ERL-GB.
Persons to Contact: N. Richards, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).
Grant/Contract Laboratory Involved and Principal Investigators: U. of West Florida, Pensacola, FL 32504, R. Rao.
Program Office Support: OEMI.
References: Not supplied.

1244 ISOGENIC FISH

Biological Activity Detected: Presumptive oncogenicity; Teratology.

Principle: Isogenic fish provide uniform progeny with predictable life stages, and an opportunity to genetically engineer genotypes for susceptability to carcinogens.

Endpoints: Qualitative: Not supplied. Quantitative: Interference with development; Melanoma/melanin proliferation.

Strengths: In-vivo; Rapid.

Weaknesses: Toxic substances may kill test animal before oncogenic/carcinogenic response is elicited; Substances may not be permeable to eggs.

Status of Development: Developmental.

Describe: Not supplied.

Applications: Water.

Samples: Pure Chemicals: PNA's, Nitrosamines, Aflotoxin, etc.

Complex Mixtures: Not supplied.

Duration: 1 month.

Cost: Not yet determined.

Interpretation: Test results indicate presumptive teratogen, presumptive carcinogen, or toxic substance.

Level of Complexity: Not supplied.

OHEE Laboratory Involved: ERL-GB.

Persons to Contact: N. Richards, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).

Grant/Contract Laboratory Involved and Principal Investigators: U. of North Carolina, Chapel Hill, NC 27514, D. Humm.

Program Office Support: OEMI.

References: Not supplied.

1245 INTEGRATED SYSTEM: DEVELOPMENT OF MUTAGEN/CARCINOGEN ACTIVATION, CONCENTRATION, SEPARATION, AND WEATHERING SYSTEMS

Biological Activity Detected: Toxicity; Mutagenicity.

Prinicple: The above procedures are being developed for use with quick screen tests. Biological, physical, and chemical methods are being used to concentrate, separate, and activate compounds which interfere with testing.

Endpoints: Qualitative: Sample concentrated, freed of interfering substances, activated, prepared for testing. Quantitative: Not supplied.

Strengths: Separation of toxic components from complex mixtures will allow detection; Concentration of dilute genotoxics will allow their detection; Weathering may assist in prediction of the environmental fate; Marine activation systems aid in predicting biological fate and food web relationships.

Weaknesses: Extensive exploratory research is required to validate the methods.

Status of Development: Developmental.

Describe: Not supplied.

Applications: Water; Food.

Samples: Pure Chemicals: PNA's; Complex Mixtures: Ambient - estuarine/marine H_2O; Energy Related - shale; Other - tissue residues.

Duration: Not supplied.

Cost: Not supplied.

Interpretation: These methods may be useful for all in-vitro and in-vivo methods to expand their application to complex samples.

Level of Complexity: Not supplied.

OHEE Laboratory Involved: ERL-GB.

Persons to Contact: N. Richards, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).

Grant/Contract Laboratory Involved and Principal Investigators: Gulf South Research Institute, E. Kline; U. of West Florida, Pensacola, FL 32504, R. Rao.

Program Office Support: OEMI; OHEE.

References: Not supplied.

1246 BIPHENYL HYDROXYLASE

Biological Activity Detected: Presumptive oncogenicity.
Principle: Pre-pre screen for carcogenicity depends on correlation of interference with hydroxylation.
Endpoints: Qualitative: Not supplied. Quantitative: Interference with enzyme reaction.
Strengths: Extremely rapid; inexpensive.
Weaknesses: Requires extensive development, modification and validation; Current data based on correlation only; Mechanism unknown.
Status of Development: Developmental.
Describe: Requires extensive development, modification, and validation.
Applications: Water; Multimedia.
Samples: Pure Chemicals: Multiple classes being screened.
Complex Mixtures: Not supplied.
Duration: Not supplied.
Cost: Not supplied.
Interpretation: After validation and development, the test may be useful as a rapid, inexpensive pre-pre screen.
Level of Complexity: 0.
OHEE Laboratory Involved: ERL-GB.
Persons to Contact: N. Richards, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).
Grant/Contract Laboratory Involved and Principal Investigators: Denver Research Institute, J. Schmidt-Coderis.
Program Office Support: OEMI.
References: Not supplied.

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1301 MAMMALIAN TERATOLOGY

Biological Activity Detected: Teratology.

Principle: Determine the potential of the compound to produce anatomical malformations.

Endpoints: Qualitative: N/A. Quantitative: Number and type of malformations.

Strengths: Biologically active compounds are readily detected; Same techniques used for most species.

Weaknesses: Only gross anatomical malformations are noted.

Status of Development: Validated.

Describe: Protocols and methods well established. Compounds are being tested on a routine basis.

Applications: Multimedia.

Samples: Pure Chemicals: All classes. Complex Mixtures: Ambient; Industrial, Energy Related; Transportation Related; Other.

Duration: 6 weeks for small rodents, start to report. Additional gestational time needed for larger animals.

Cost: $6,000/specie/compound.

Interpretation: The teratogenic potential of compounds can be established.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Toxic Effects Branch, Perinatal Toxicology Section.

Persons to Contact: K.D. Courtney, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2370).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OPP.

References: 1) OPP Guidelines for Teratology.

1302 PERINATAL TOXICOLOGY

Biological Activity Detected: Postnatal development.

Principle: Establish biochemical markers to determine normalacy of postnatal development. Isozyme profiles of lactic dehydrogenase (LDH) and creative phosphokinase (CPK) show definite developmental patterns from day 7 to 14 in the postnatal mouse.

Endpoints: Qualitative: N/A. Quantitative: LDH and CPK total activities and isozyme profiles are determined in postnatal mice after prenatal and/or actational exposure. Results are entered into computer by key punch cards and analyzed by established program.

Strengths: Establish a measurable parameter of postnatal development that can be quantified; Determining the cardiac isozymes permits correlation with clinical human data; Comparative species studies use same techniques and interpretations.

Weaknesses: Must wait for animals to be born.

Status of Development: Validated.

Describe: The postnatal isozyme profiles of LDH and CPK have been established and treated animals are being evaluated.

Applications: Multimedia.

Samples: Pure Chemicals: All classes. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other.

Duration: Gestation plus 4 weeks, start to report.

Cost: $3,000/compound.

Interpretation: Postnatal development is evaluated with the same techniques that are used in human medicine so that results are directly comparable.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Toxic Effects Branch, Perinatal Toxicology Section.

Persons to Contact: K.D. Courtney, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2370).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OPP.

References: Not yet available.

1303 FETAL TOXICITY IN RATS, MICE, GUINEA PIGS/HAMSTERS

Biological Activity Detected: Fetal toxicity including teratogenicity.

Principle: Pregnant females are treated with chemical (or other agent) during the period of major organogenesis of their litters. The animals are sacrificed before term and the fetuses subsequently examined for signs of toxicity including visual and skeletal defects.

Endpoints: Qualitative: Descriptions of defects and anomalies encountered. Quantitative: Degree of fetal toxicity, and incidence of defects encountered.

Strengths: A fairly rapid test, 30 to 45 days to final report; Much historical background.

Weaknesses: Difficulty in assessing form and degree of fetal toxicity; Difficulty in extrapolation of data to human species.

Status of Development: Validated.

Describe: Standardized protocols have been available for over 10 years.

Applications: Multimedia.

Samples: Pure Chemicals: All classes. Complex Mixtures: Drinking water contaminants.

Duration: 45 days from initiation of treatment to completion of analysis.

Cost: $10,000/species.

Interpretation: A positive response in a species that has some metabolic similarities to man would suggest that the compound or mixture in question has the potential to induce abnormal development in humans.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Biology Division, Neurobiology Branch.

Persons to Contact: N. Chernoff, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2326); R. Kavlock, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2326).

Grant/Contract Laboratory Involved and Principal Investigators: Contract currently under negotiation for the testing of 5 pesticides/year by established protocols.

Program Office Support: OPP; ORD.

References: 1) Pesticide Registration Guidelines. In preparation. 2) Testing of Chemicals for Carcinogenicity, Mutagenicity, and Teratogenicity. Published by Minister of Health and Welfare, Canada, 1973.

1304 DEVELOPMENTAL TOXICITY IN NEONATAL RATS

Biological Activity Detected: Developmental toxicity.

Principle: Pregnant rats are treated with compounds from the time of implantation continuing through lactation. The neonatal rats are then examined for a number of developmental milestones. This design allows for the continuous exposure of a mammalian organism through its most sensitive periods of development.

Endpoints: Qualitative: Growth and viability of neonatal rats. Quantitative: Measurement of developmental milestones in early postnatal life including reflex and morphological development; Also measurement of open field behavior in young adults.

Strengths: Exposure to organisms during the perinatal period maximizes the possibility of producing alterations in the morphological and behavioral aspects of the exposed animal.

Weaknesses: Testing is a labor-intensive operation; Lack of standardized procedures by various investigations; Difficult to extrapolate to humans.

Status of Development: Developmental.

Describe: Investigators in this field are evaluating the reliability and sensitivity of various test procedures.

Applications: Air; Water; Food.

Samples: Pure Chemicals: All classes. Complex Mixtures: N/A.

Duration: 3 to 4 months, start to report.

Cost: $10,000/species.

Interpretation: The production of growth disturbances/behavioral anomalies in the young postnatal animal is one of the more sensitive indicators of developmental toxicity. The implication of this test is that positive results may point to the induction of behavioral disturbances in humans subsequent to perinatal exposure. This link-up to human effects, however, has yet to be empirically demonstrated.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Biology Division, Neurobiology Branch.

Persons to Contact: R. Kavlock, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2326); N. Chernoff, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2326).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OPP; ORD.

References: 1) Wiess, B., and J. Spyker. Behavioral Implications of Prenatal and Early Postnatal Exposure to Chemical Pollutants. Pediatrics, 53(5):Part III, May 1974. 2) Final Report of the Committee on Postnatal Evaluation of Animals Subjected to Insult During Development. C. Kimmel, Chairperson. Funded by the Nat. Inst. of Envir. Hlth. Sci., Mar. 1977.

1305 TERATOLOGY IN-VIVO/IN-VITRO

Biological Activity Detected: Teratology; Toxicology.

Principle: Treat pregnant rats from day 6 to 9 of gestation, then remove embryos and grow them in tissue culture for 24 to 48 hrs. During the culture interval, determinations of anatomical and biochemical growth are made.

Endpoints: Qualitative: Determine degree and normalacy of neuralation and somite development; Quantitative: Measure growth indicators, DNA, and protein.

Strengths: The maternal animal is treated; The embryo is accessible for a long enough period to make some measurements; Zero time measurements can use embryos from the same litter; Delays in growth during this gestational stage could result in malformations or abnormal development such as extra ribs; Embryos from the same litter are available for residue determination.

Weaknesses: The culture of the embryos is limited to 48 hours.

Status of Development: Being implemented.

Describe: Embryos from treated animals are being grown in culture. Methods for growing control embryos have been established.

Applications: Multimedia.

Samples: Pure Chemicals: All classes. Complex Mixtures: Ambient; Industrial; Energy Related; Transportation Related; Other.

Duration: 4 weeks, start to report.

Cost: $2,000/compound.

Interpretation: Toxic as well as teratogenic evaluators are determined for the embryo.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-RTP, Environmental Toxicology Division, Toxic Effects Branch, Perinatal Toxicology Section.

Persons to Contact: K.D. Courtney, U.S. EPA, HERL-RTP, Research Triangle Park, NC 27711, (FTS 629-2370).

Grant/Contract Laboratory Involved and Principal Investigators: U. of Michigan, School of Medicine, Department of Anatomy, Ann Arbor, MI 48104, A. Beaudoin.

Program Office Support: OTS.

References: Not yet available.

1306 DIRECT SPECTRAL MEASUREMENT OF THE BIOCHEMICAL DEVELOPMENT OF THE NERVOUS SYSTEM

Biological Activity Detected: Delayed and arrested development.

Principle: A large change in the capacity of the nervous system for oxidative metabolism occurs with development. This increase may be followed directly in slices of tissue by taking advantage of the known spectral properties of the cytochromes using dual-wavelength spectroscopy. During brain development there are quantitative and qualitative differences in the way the tissue will respond to stimulation (e.g., electrical, elevated K$^+$) metabolically that may be observed using polarographic, spectral, and enzymatic analyses.

Endpoints: Qualitative: Developmental changes in: Cytochrome levels; Oxygen uptake in response to stimulation; Lactic acid output; Uptake and release of neurotransmitters; Amino acid concentrations; Redox changes produced in tissue pyridine nucleotides, flavoproteins, and cytochromes in response to standard stimulants.

Quantitative: Measurements of: Cytochrome levels; Oxygen uptake in response to stimulation; Lactic acid output; Uptake and release of neurotransmitters; Amino acid concentrations; Redox changes produced in tissue pyridine nucleotides, flavoproteins, and cytochromes in response to standard stimulants.

Strengths: Spectral measurements can be applied to as little as 3 mg of tissue; Responses of tissues dependent upon the integrity of a wide variety of systems within the tissue (e.g., cell excitability, Na$^+$, K$^+$-ATPase, neurotransmittance, uptake, release and intrinsic activity, glycolytic and TCA cycle enzymes, etc.). The responses to K$^+$ are multiphasic, one phase probably being applicable to neuronal responses, the other to glial responses. Consequently, the system is unique in that it will detect a wide variety of types of damage.

Weaknesses: Principle weakness is that it does not lend itself to immediate identification of mechanism unless there is a direct effect on energy metabolism proper so that crossovers can be identified. However, this disadvantage is overcome by the fact that delays in development resulting from early exposure to Pb can be almost perfectly correlated with delays in morphological development (e.g., synaptogenesis).

Status of Development: Being implemented.

Describe: The basic developmental patterns for the cerebral cortex have been established for the spectral measurements of cytochrome concentrations and form of the metabolic responses. Spectral changes induced by K$^+$ have been correlated with respiratory changes, lactic acid output, and changes in tissue adenine nucleontides. Delays in development have been demonstrated in these parameters with Pb and correlated with delayed synaptogenesis in the rat.

Applications: Multimedia.

Samples: Pure Chemicals: Organics; Inorganics. Complex Mixtures: Industrial; Energy Related; Transportation Related; Other.

1306 DIRECT SPECTRAL MEASUREMENT OF THE BIOCHEMICAL DEVELOPMENT OF THE NERVOUS SYSTEM (continued)

Duration: As short as 3 weeks to as long as 3 months if animals are to be taken to sexual maturity.

Cost: $2,500 to $25,000, depending upon duration and number of replications.

Interpretation: Delays in biochemical development of the brain have been well correlated with delays in morphological development (synaptogenesis) and behavioral development (activity away from mother during lactation) in lead-treated animals. With these data as support, delayed biochemical development may be interpreted as presumptive evidence of retarded mental development.

Level of Complexity: 3.

OHEE Laboratory Involved: HERL-CIN, Laboratory Studies Division, Toxicological Assessment Branch.

Persons to Contact: R.J. Bull, U.S. EPA, HERL-CIN, 26 W. St. Clair St., Cincinnati, OH 45268, (FTS 684-7213).

Grant/Contract Laboratory Involved and Principal Investigators: In-house.

Program Office Support: OHEE.

References: 1) Himwich, W.A. Developmental Neurobiology. C.C. Thomas, Springfield, IL, 1970. pp. 22-46, 311-330, 331-369, 370-392. 2) Bull, R.J., and S.D. Lutkenhoff. J. Neurochem., 21:913-922, 1973. 3) Bull, R.J., and J.T. Cummins. J. Neurochem., 21:923-937, 1973. 4) Bull, R.J., and J.J. O'Neill. Psychopharmacol. Commun., 1:109-115, 1975. 5) Bull, R.J., P.M. Stanaszek, and S.D. Lutkenhoff. Envir. Hlth. Perspect., 12: 89-95, 1975. 6) Bull, R.J. J. Neurochem., 26:149-156, 1976.

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2101 FRESHWATER ALGAL ASSAY BOTTLE TEST

Biological Activity Detected: Toxicity; Stimulation.

Principle: Standard test alga (*Selenastrum capricornutum*) is added to a test water, cultured under standard light, temperature, and gas exchange conditions, and evaluated for inhibitory (toxic) or stimulatory response to pollutant stress.

Endpoints: Qualitative: Can be used to screen stimulatory or inhibitory properties of point and non-point source pollutants. Quantitative: Response in mg dry wt l^{-1} of test alga either stimulatory (% S_{14}) or inhibited (% I_{14}) at day 14. Results are expressed as % stimulation or % inhibition as compared to control vs waste concentration.

Strengths: Detection of bioreactive components in a test water, or of waste discharge; identification of toxic or stimulatory components.

Weaknesses: Insufficient application of test to relate to potential health effects of a pollutant.

Status of Development: Validated.

Describe: N/A.

Applications: Water.

Samples: Pure Chemicals: Nutrients, NO_3, NO_2, NH_3, Ortho-P, Tot-P, Heavy metals, Pesticides, Herbicides, Insecticides. Complex Mixtures: Ambient - receiving water; Industrial - waste discharges; Energy Related - coal storage, leachates; Other - new product formulations, i.e., detergents.

Duration: Test: 14 days; Analysis: 5 days; Total: 21 days maximum in most cases.

Cost: $400 to evaluate a compound or complex waste.

Interpretation: Test data can be used to define nutrient limitation, heavy metal toxicity, and inhibitory or stimulatory properties of complex wastes, as they effect ecology of aquatic systems.

Level of Complexity: 1.

OHEE Laboratory Involved: ERL-COR, Assessment Criteria Development Division, Special Studies Branch; ERL-DUL, Newtown Fish Toxicology Station.

Persons to Contact: W.E. Miller, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4775, Commercial 503 757-4775); T. Shiroyama, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4776, Commercial 503 757-4776); J.C. Greene, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4764, Commercial 503 757-4764); E. Robinson, U.S. EPA, ERL-DUL, Newtown Fish Toxicology Station, 3411 Church St., Cincinnati, OH 45244, (FTS 684-8601).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OTS.

References: 1) Algal Assay Procedure Bottle Test. U.S. EPA, Aug. 1971. 2) Standard Methods for the Examination of Water and Wastewater. 14th edition, 1975. To be published by ASTM.

2102 MARINE ALGAL ASSAY BOTTLE TEST

Biological Activity Detected: Toxicity; Stimulation.

Principle: Standard test algae are added to a test water, cultured under standard light, temperature, and gas exchange conditions and evaluated for inhibitory (toxic) or stimulatory response to pollutant stress.

Endpoints: *Qualitative*: Can be used to screen potential stimulatory or toxic properties of pollutants. *Quantitative*: Response in mg dry wt l^{-1} of the stimulatory (% S_{14}) or inhibitory (% I_{14}) response as compared to control vs control to which a waste concentration has been added.

Strengths: Detection of stimulatory and/or inhibitory bioreactive components in a test water, and of waste discharge.

Weaknesses: Insufficient application of test (round Robin); Inadequate to predict possible health effects of specific pollutants.

Status of Development: Validated.

Describe: Interlaboratory calibration is necessary, as are broad application studies, i.e., complex wastes, organic compounds, etc.

Applications: Water.

Samples: *Pure Chemicals*: Nutrients, i.e., Nitrogen and Phosporous, Heavy metals, PCB's, Chloramines, Free chlorine. *Complex Mixtures*: Ambient - receiving waters; Industrial - waste discharges, dredge spoil, monochlorinated organics detergent builders.

Duration: Test: 14 days; Analysis: 5 days; Total: 21 days.

Cost: $400 to evaluate a compound of complex waste.

Interpretation: Test data can be used to define nutrient limitation, heavy metal toxicity, and inhibitory or stimulatory properties of complex wastes, as they effect ecology of marine ecosystems.

Level of Complexity: I.

OHEE Laboratory Involved: ERL-NAR, Toxicology Branch, Marine Toxicology Team; ERL-COR, Marine and Freshwater Branch; ERL-GB, Experimental Environments Branch.

Persons to Contact: *J. Gentile*, U.S. EPA, ERL-NAR, South Ferry Rd., Narragansett, RI 02882, (FTS 838-4843 X244); *D. Specht*, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4675); *G. Walsh*, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561 (FTS 686-9011).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OTS.

References: 1) Marine Algal Assay Procedure Bottle Test. U.S. EPA, Dec. 1974. Bioassay Procedures for the Ocean Disposal Permit Program. EPA-600/9-78-010, U.S. EPA, 1978.

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2211 ACUTE FISH TOXICITY TEST, STATIC AND FLOW-THROUGH, ALL FRESHWATER SPECIES*

Biological Activity Detected: Toxicity.

Principle: Determine the 96-hr LC50 of various pure compounds and complex mixtures on freshwater organisms.

Endpoints: Qualitative: Behavior. Quantitative: Mortality (LC50).

Strengths: A "hard" number on lethality.

Weaknesses: The LC50 is not protective of aquatic species. Therefore, one must estimate or test for lower, no-effect concentrations.

Status of Development: Validated.

Describe: The 96-hr flow-through and static aquatic toxicity tests have been conducted for many years with some cross validation between laboratories; Some methods were written by Standard Methods and ASTM.

Applications: Water.

Samples: Pure Chemicals: Insecticides, Herbicides, Heavy Metals, PCB's. Complex Mixtures: Industrial - effluents; Energy Related - drilling muds, oils.

Duration: Test: 96 hours; Analysis: 96 hours.

Cost: Static test: $300; Flow-through test: $650.

Interpretation: From the tests the lethal effects of a toxicant along with a statistically valid 95% confidence interval can be determined.

Level of Complexity: I.

OHEE Laboratory Involved: ERL-DUL, Technical Assistance Branch.

Persons to Contact: C. Stephan, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804 (FTS 783-9510, Commercial 218 727-6692 X570).

Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, Inc., Wareham, MA, S. Sauter and K.J. Macek.

Program Office Support: OHEE; ORD.

References: 1) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. EPA-600/3-75-009, U.S. EPA, 1975.

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*This test is also applied to marine fish. See 2311.

2212 SUBCHRONIC EMBRYO-LARVAL, EARLY JUVENILE FISH TOXICITY TEST

Biological Activity Detected: Toxicity.

Principle: This test involves a 30-day exposure of the embryo-larval, early juvenile stages of development.

Endpoints: Qualitative: Behavior. Quantitative: Survival; Growth; Deformities; Determination of an estimated maximum acceptable toxicant concentration (MATC).

Strengths: Direct measure of an MATC; Gives an excellent estimate of chronic toxicity in 1 month. Chronic tests themselves would require 12 months to complete.

Weaknesses: Requires 30 days to complete test; Requires a supply of fish embryos.

Status of Development: Validated.

Describe: These tests have been run successfully by several contract laboratories as well as routinely by several EPA laboratories.

Applications: Water.

Samples: Pure Chemicals: Inorganics, Organics. Complex Mixtures: Industrial; Energy Related.

Duration: Test: 30 days; Analysis: Included in the 30 days.

Cost: $6,000/test.

Interpretation: This test gives an excellent estimate of the chronic (life-cycle) toxicity of individual toxicants or complex mixtures to fish.

Level of Complexity: 2.

OHEE Laboratory Involved: ERL-DUL, Research Branch, Physiological Effects of Toxicants Section.

Persons to Contact: J.M. McKim, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9567, Commercial 218 727-6692 X567); W.A. Brungs, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9546, Commercial 218 727-6692 X546).

Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, Inc., Wareham, MA, S. Sauter and K.J. Macek.

Program Office Support: OHEE; ORD.

References: 1) McKim, J.M. Evaluation of Tests with the Early Life-Stage of Fish for Predicting Long-Term Toxicity. J. Fish Res. Bd. Can., 34(8):1148-1154, 1977. 2) U.S. EPA. Proposed Recommended Bioassay Procedure for Egg and Fry Stages of Freshwater Fish (manuscript). U.S. EPA, Duluth, MN. 3) Sauter, S., K.S. Buxton, K.J. Macek, and S.F. Petrocelli. Effects of Continuous Exposure to Lead, Chromium, Copper, and Cadmium on Eggs and Fry of Selected Freshwater Fish. Ecol. Res. Series, U.S. EPA, Duluth, MN, 1976.

2213 CHRONIC FISH TOXICITY TEST, AMERICAN FLAGFISH (*JORDANELLA FLORIDAE*)

Biological Activity Detected: Toxicity.

Principle: Determine the impact of toxicants on survival, growth, and reproduction of a freshwater fish with a rapid life cycle.

Endpoints: *Qualitative*: Behavior. *Quantitative*: Growth; Survival; Reproduction success (fecundity, hatchability); Determination of a maximum acceptable toxicant concentration (MATC).

Strengths: Similar in sensitivity to commonly tested freshwater fish, i.e., brook trout and fathead minnow; Short life cycle compared to most fish; Data generated on all stages of life cycle including those stages considered to be most sensitive.

Weaknesses: Fish is semitropical and may not have direct application to most U.S. waters.

Status of Development: Validated.

Describe: Many people at ERL-DUL have run this test successfully.

Applications: Water.

Samples: *Pure Chemicals*: Pesticides, Metals. *Complex Mixtures*: Waste oil.

Duration: Test: 6 months; Analysis: Included in 6 months.

Cost: $16,000.

Interpretation: This test can be used as an indicator of potential chronic fish effects.

Level of Complexity: 3.

OHEE Laboratory Involved: ERL-DUL, Research Branch, Physiological Effects of Toxicants Section.

Persons to Contact: *W.A. Brungs*, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9546, Commercial 218 727-6692 X546); *R. Spehar*, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9521, Commercial 218 727-6692 X521).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; ORD.

References: 1) Smith, W.E. A Cyprinodont Fish, *Jordanella floridae*, as a Reference Animal for Rapid Chronic Bioassays. J. Fish. Res. Bd. Can., 39:329-330, 1973. 2) Spehar, R.L. Cadmium and Zinc Toxicity to *Jordanella floridae*. J. Fish. Res. Bd. Can., 33:1939-1945, 1976. 3) U.S. EPA Committee on Aquatic Bioassays. Recommended Bioassay Procedures for Brook Trout, Bluegill, Fathead Minnow, and Flagfish Chronic Tests. U.S. EPA, Duluth, MN, 1972.

2214 FISH RESPIRATORY ACTIVITY TOXICITY TEST, ELECTRODE CHAMBER METHOD

Biological Activity Detected: Toxicity.
Principle: Bioelectric signals generated by respiratory activities are electronically amplified and recorded on stripchart records.
Endpoints: Gill purge (cough) and ventilation rates. Qualitative: Behavior. Quantitative: Rate of increase/unit of time.
Strengths: Rapid; Predictive.
Weaknesses: Stripchart records must be interpreted.
Status of Development: Being implemented.
Describe: The test results have been published (see references) and other researchers are starting to use this method.
Applications: Water.
Samples: Pure Chemicals: Multi. Complex Mixtures: Industrial.
Duration: Test: 4 days; Analysis: 1 day.
Cost: $750/chemical.
Interpretation: Concentrations which do not cause a statistically significant increase in gill purge rates are not likely to cause long-term adverse effects.
Level of Complexity: 1.
OHEE Laboratory Involved: ERL-DUL, Research Branch, Physiological Effects of Pollutants Section.
Persons to Contact: R. Drummond, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9511, Commercial 218 727-6692 X511; R. Carlson, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9591, Commercial 218 727-6692 X591).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OHEE.
References: 1) Spoor, W.A., et al. Trans. Am. Fish. Soc., 1971. 2) Drummond, R., et al. J. Fish. Res. Bd. Can., 1973. 3) Drummond, R., et al. Trans. Am. Fish. Soc., 1974. 4) Carlson, R., and R. Drummond. Water Res., 1978. 5) Drummond, R., and R. Carlson. Ecol. Rep. Series, U.S. EPA, 1978.

2215 FISH AVOIDANCE TEST, GRADIENT TANKS

Biological Activity Detected: Toxicity; Physical environment.
Principle: Levels of environmental variables and toxicants avoided by fish are determined.
Endpoints: Qualitative: Avoidance behavior. Quantitative: Locomotor activity/unit of time.
Strengths: Short-term tests; Endpoints easy to determine; Collection of data can be automated.
Weaknesses: Needs validation.
Status of Development: Developmental.
Describe: Laboratory investigation underway.
Applications: Water.
Samples: Pure Chemicals: Dissolved gases, Oxygen, Metals, Organics.
Complex Mixtures: Industrial; Energy Related.
Duration: Test: 4 hours; Analysis: 1 hour.
Cost: $150.
Interpretation: Avoidance has ecological significance.
Level of Complexity: 1.
OHEE Laboratory Involved: ERL-DUL, Office of the Director.
Persons to Contact: W.A. Spoor, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9506, Commercial 218 727-6692 X506).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: ORD.
References: 1) Spoor, W.A., and R. Drummond. Trans. Am. Fish. Soc., 101:714-715, 1972.

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2221 ACUTE INVERTEBRATE TOXICITY TEST, STATIC AND FLOW-THROUGH, ALL FRESHWATER SPECIES*

Biological Activity Detected: Toxicity.

Principle: Determine the 48-hr LC50 or EC50 of various pure compounds and complex mixtures on freshwater organisms.

Endpoints: Qualitative: Behavior. Quantitative: Mortality (LC50) or Immobilization EC50.

Strengths: A "hard" number on lethality, or measurable effect is determined.

Weaknesses: The LC50 or EC50 is not protective of aquatic species. Therefore, one must estimate or test for lower, no-effect concentrations.

Status of Development: Validated.

Describe: The 48-hr and 96-hr flow-through and static aquatic toxicity tests have been conducted for many years. Some methods were written by Standard Methods and ASTM.

Applications: Water.

Samples: Pure Chemicals: Insecticides, Herbicides, Heavy Metals, PCB's. Complex Mixtures: Industrial - effluents; Energy Related - drilling muds, oils.

Duration: Test: 48 or 96 hours; Analysis: 48 hours.

Cost: Static test: $300; Flow-through test: $650.

Interpretation: From the tests, the lethal effects of a toxicant along with a statistically valid 95% confidence interval can be determined.

Level of Complexity: I.

OHEE Laboratory Involved: ERL-DUL, Technical Assistance Branch; ERL-DUL, Extramural Program Branch.

Persons to Contact: C. Stephan, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804 (FTS 783-9510, Commercial 218 727-6692 X570); K.E. Biesinger, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9524, Commercial 218 727-6692 X524).

Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, Inc., Wareham, MA, S. Sauter and K.J. Macek.

Program Office Support: OHEE; ORD.

References: 1) Biesinger, K.E., and G. Christensen. Effects of Various Metals on Survival, Growth, Reproduction, and Metabolism of *Daphnia magna*. J. Fish Res. Bd. Can., 29(2):1691-1700, 1972. 2) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. EPA-600/3-75-009, U.S. EPA, 1975.

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*This test is also applied to marine invertebrates. See 2321.

2222 SUBCHRONIC INVERTEBRATE TOXICITY TEST, STREAM INSECTS

Biological Activity Detected: Toxicity.

Principle: The insects are exposed for 4 weeks. The time length allows for a good estimate of toxicity.

Endpoints: Qualitative: Behavior. Quantitative: Survival LC50; Bioaccumulation.

Strengths: Allows testing of stream invertebrates.

Weaknesses: Requires a "clean" collecting site near test facility; Requires good water source since it is a flow-through system.

Status of Development: Developmental.

Describe: System is developed and can be used if the need was established (i.e., could be implemented but isn't).

Applications: Water.

Samples: Pure Chemicals: Heavy metals, Pesticides. Complex Mixtures: Not tested.

Duration: Test: 4 week exposures; Analysis: Could be included in test time.

Cost: $2,800 at 4 weeks at $35,000/manyear.

Interpretation: It is a procedure which allows exposure for a time longer than acute (4-day) tests. This allows a better prediction of toxic effects.

Level of Complexity: 1 to 2.

OHEE Laboratory Involved: ERL-DUL, Research Branch, Physical Pollutant Section.

Persons to Contact: R.L. Anderson, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9565, Commercial 218 727-6692 X565).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; ORD.

References: 1) Spehar, R.L., R.L. Anderson, and J.T. Fiandt. Toxicity and Bioaccumulation of Cadmium and Lead in Aquatic Invertebrates. Envir. Pollut., 15:195, 1978. 2) Anderson, R.L., and D. DeFoe. Toxicity and Bioaccumulation of Endrin and Methoxychlor by Aquatic Invertebrates. 1979. In press.

2223 SUBCHRONIC INVERTEBRATE TOXICITY TEST, CHIRONOMID (TANYTARSUS DISSIMILIS)

Biological Activity Detected: Toxicity.

Principle: The animals are exposed from egg to 2nd or 3rd instar for 10 to 12 days of total exposure.

Endpoints: Qualitative: N/A. Quantitative: Survival; Growth; LC50 and EC values.

Strengths: Exposure through molting; Can also measure growth effects.

Weaknesses: Static, has only been tested with metals.

Status of Development: Developmental.

Describe: A report is being prepared regarding exposure to cadmium, lead, copper, and zinc.

Applications: Water.

Samples: Pure Chemicals: Heavy metals. Complex Mixtures: N/A.

Duration: Test: 10 to 12 days exposure; Analysis: Up to 2 weeks including preparation and clean-up.

Cost: 2 weeks at $35,000/manyear equals approximately $1,400; Chemical analysis might be additional if complex compounds were used.

Interpretation: The system exposes an insect during embryogenesis, hatching, growth, and molting. These 4 events are critical to the survival of the animal.

Level of Complexity: 2 to 3

OHEE Laboratory Involved: ERL-DUL, Research Branch, Physical Pollutant Section.

Persons to Contact: R.L. Anderson, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9565, Commercial 218 727-6692 X565).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; ORD.

References: None, in-house development.

2224 CHRONIC INVERTEBRATE TOXICITY TEST, WATER FLEA (DAPHNIA MAGNA)

Biological Activity Detected: Toxicity; Reproduction.
Principle: Animals are exposed for 3 to 4 weeks. The exposure period includes molting and reproduction.
Endpoints: Qualitative: N/A. Quantitative: Survival (LC50); Reproduction.
Strengths: Low equipment cost; Manpower requirements.
Weaknesses: Renewal system.
Status of Development: Being implemented.
Describe: Procedure has been published and is now being used with variation in many places.
Applications: Water.
Samples: Pure Chemicals: Metals, Organics. Complex Mixtures: Industrial; Energy Related.
Duration: Test: 3 to 4 weeks may be a good estimate. This would include data analysis and perhaps chemical analysis if single or low number mixtures are used.
Cost: 3 weeks at $35,000/manyear equals approximately $2,100.
Interpretation: Test allows fairly rapid screening of pollutants with a zooplankton representative.
Level of Complexity: 2.
OHEE Laboratory Involved: ERL-DUL, Technical Assistance Branch.
Persons to Contact: C. Stephan, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9510, Commercial 218 727-6692 X510).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OHEE; ORD.
References: 1) Biesinger, K., and G. Christensen. Effects of Various Metals on Survival, Growth, Reproduction and Metabolism of *Daphnia magna*. J. Fish. Res. Bd. Can., 29(12):1691-1700, 1972. 2) ASTM DRAFT. Proposed Standard Practice for Conducting Life-Cycle Toxicity Tests with the Daphnid, *Daphnia magna*.

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2231 ACUTE PLANT TOXICITY TEST, DUCKWEED (LEMNA MINOR)

Biological Activity Detected: Toxicity; Residue.
Principle: Exposure of growing plants for 1 week.
Endpoints: Qualitative: N/A. Quantitative: Growth as reflected in frond count; EC values.
Strengths: Only flow-through system available for aquatic plants; Fast.
Weaknesses: Not developed to a point where weakness can be adequately described.
Status of Development: Developmental.
Describe: Exposure to copper has been completed. Exposure with other compounds is projected. Completion of procedure is projected for 1979.
Applications: Water.
Samples: Pure Chemicals: Metals, Organics. Complex Mixtures: Industrial; Energy Related.
Duration: Test: 1 week exposure, perhaps 1 to 2 weeks for preparation and clean-up.
Cost: 2 weeks at $35,000/manyear equals approximately $1,400; Analysis cost may be included if simple compounds are used.
Interpretation: This is the only system which allows exposure of an aquatic plant. Toxicity and bioaccumulation data should be obtainable from the procedure.
Level of Complexity: 1.
OHEE Laboratory Involved: ERL-DUL, Research Branch, Physical Pollutant Section.
Persons to Contact: R.L. Anderson, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9565, Commercial 218 727-6692 X565).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OHEE; ORD.
References: 1) Walbridge, C.T. A Flow-Through Testing Procedure with Duckweed (*Lemna minor*). Ecol. Res. Series, EPA 600/3-77-108, U.S. EPA, 1977.

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2241 LABORATORY ECOSYSTEM TEST

Biological Activity Detected: Toxicity.

Principle: To determine the effects of toxicants on ecosystem processes.

Endpoints: Qualitative: N/A. Quantitative: Modifications in rates and components of organic carbon budget includes water inflow and outflow; System photosynthesis and respiration; Periphyton growth; Benthic macroinvertebrates; Sediment accumulation and degradation; Macrophyte decomposition; MATC, based on change in the ecosystem processes.

Strengths: More realistic than single species testing; Includes species and environment interaction; Includes impact of environment on toxicant; Can follow accumulation of toxicants in food web; Includes some organisms not traditionally included in freshwater toxicology.

Weaknesses: Labor intensive; Difficult to obtain degree of replication necessary for statistical treatment of data; Complex interactions make data evaluation and interpretation difficult; Must extrapolate to natural situations.

Status of Development: Developmental.

Describe: Development of preliminary non-toxicant methods has just been initiated.

Applications: Water.

Samples: Pure Chemicals: Inorganics, Organics. Complex Mixtures: N/A.

Duration: Test: 3 to 5 months; Analysis: An additional 2 to 3 months.

Cost: $35,000.

Interpretation: Data can be used to identify sensitive ecosystem processes as well as to determine potential assimilation capacity.

Level of Complexity: 3.

OHEE Laboratory Involved: ERL-DUL, Newtown Fish Toxicology Station.

Persons to Contact: S.F. Hedtke, U.S. EPA, ERL-DUL, Newtown Fish Toxicology Station, 3411 Church Street, Cincinnati, OH 45244, (FTS 684-8601).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: ORD.

References: Work is presently in developmental stages. Information on test system has not been published. See Persons to Contact.

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2311 ACUTE FISH TOXICITY TEST, STATIC AND FLOW-THROUGH, ALL MARINE SPECIES*

Biological Activity Detected: Toxicity.

Principle: Determine the 96-hr LC50 of various pure compounds and complex mixtures on marine organisms.

Endpoints: Qualitative: Behavior. Quantitative: Mortality (LC50).

Strengths: A "hard" number on lethality.

Weaknesses: The LC50 is not protective of aquatic species. Therefore, one must estimate or test for lower, no-effect concentrations.

Status of Development: Validated.

Describe: The 96-hr flow-through and static aquatic toxicity tests have been conducted for many years with cross validation by many laboratories; Some methods were written by Standard Methods and ASTM.

Applications: Water.

Samples: Pure Chemicals: Insecticides, Herbicides, Heavy Metals, PCB's. Complex Mixtures: Industrial - effluents; Energy Related - drilling muds, oils.

Duration: Test: 96 hours; Analysis: 96 hours.

Cost: Static test: $300; Flow-through test: $650.

Interpretation: From the tests the lethal effects of a toxicant along with a statistically valid 95% confidence interval can be determined.

Level of Complexity: I.

OHEE Laboratory Involved: ERL-DUL, Technical Assistance Branch.

Persons to Contact: C. Stephan, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804 (FTS 783-9510, Commercial 218 727-6692 X570).

Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, Inc., Wareham, MA, S. Sauter and K.J. Macek.

Program Office Support: OHEE; ORD.

References: 1) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. EPA-600/3-75-009, U.S. EPA, 1975.

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*This test is also applied to freshwater fish. See 2211.

2312 SUBCHRONIC EMBRYO-LARVAL FISH TOXICITY TEST, SHEEPSHEAD MINNOW (*CYPRINODON VARIEGATUS*)

Biological Activity Detected: Toxicity; Growth; Pathologic effects.
Principle: To determine the effects of a toxicant on the early life stages of the sheepshead minnow.
Endpoints: Determine concentrations of a toxicant which affect survival, growth, behavior, and pathologic effects. *Qualitative*: Behavior. *Quantitative*: LC50 values; Significant differences can be established from experimental and control survival, and growth; Determination of an estimated maximum acceptable toxicant concentration (MATC).
Strengths: A good estimate of toxicity, particularly chronic toxicity, can be made in many instances.
Weaknesses: Duration is generally 28 days, or more.
Status of Development: Being implemented.
Describe: Embryo/fry studies have been conducted by ERL-GB and a private laboratory.
Applications: Water.
Samples: *Pure Chemicals*: Insecticides, Herbicides, PCB's, Pentachlorophenol. *Complex Mixtures*: N/A.
Duration: Test: 28 days; Analysis: 28 to 40 days if a chemical analysis is required for bioconcentration.
Cost: $6,000 to $7,000, depending upon whether or not chemical analyses are required.
Interpretation: From these tests the concentrations of a pollutant that affects survival, growth, etc. of a sensitive life stage of an estuarine fish can be determined.
Level of Complexity: 2.
OHEE Laboratory Involved: ERL-GB, Experimental Environments Branch.
Persons to Contact: *D.J. Hansen*, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).
Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, EG & G, Rt. 6, Box 1002, Pensacola, FL 32507, P.R. Parrish.
Program Office Support: OHEE; OPP; OWHM.
References: 1) Schimmel, S.C., P.R. Parrish, D.J. Hansen, J.M. Patrick, Jr., and J. Forester. Endrin: Effects on Several Estuarine Organisms. Proc. 28th Annu. Conf. Southeast. Asso. Game Fish Comm., 1974.

2313 CHRONIC FISH TOXICITY TEST, SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS)

Biological Activity Detected: Toxicity; Growth; Pathologic effects; Fecundity.

Principle: To determine the chronic effects of a pollutant on an estuarine fish.

Endpoints: Qualitative: Behavior. Quantitative: Growth; Survival; Fecundity; Pathologic effects.

Strengths: A good estimate of toxic affects.

Weaknesses: Cost; Time.

Status of Development: Being implemented.

Describe: Both ERL-GB and Bionomics, Pensacola, a private laboratory, have conducted the tests, although both laboratories have not completed full chronic tests on the same chemical for comparison.

Applications: Water.

Samples: Pure Chemicals: Insecticides, Herbicides. Complex Mixtures: N/A.

Duration: Test: 4 to 5 months; Analysis: 6 to 7 months.

Cost: $35,000 with analytical back-up.

Interpretation: One of the best available estimates of the effects of a pollutant on an estuarine fish is obtained.

Level of Complexity: 3.

OHEE Laboratory Involved: ERL-GB, Experimental Environments Branch.

Persons to Contact: D.J. Hansen, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).

Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, EG & G, Rt. 6, Box 1002, Pensacola, FL 32507. P.R. Parrish.

Program Office Support: OHEE; OPP; OWHM.

References: 1) Hansen, D.J., S.C. Schimmel, and J. Forester. Endrin: Effects on the Entire Life Cycle of a Salt Water Fish. J. Toxicol. Envir. Hlth., 3:721-733, 1977.

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2321 ACUTE INVERTEBRATE TOXICITY TEST, STATIC AND FLOW-THROUGH, ALL MARINE SPECIES*

Biological Activity Detected: Toxicity.

Principle: Determine the 48-hr LC50 or EC50 of various pure compounds and complex mixtures on marine organisms.

Endpoints: Qualitative: Behavior. Quantitative: Mortality (LC50) or EC50.

Strengths: A "hard" number on lethality, or measurable effect, is determined.

Weaknesses: The LC50 or EC50 is not protective of aquatic species. Therefore, one must estimate or test for lower, no-effect concentrations.

Status of Development: Validated.

Describe: The 48-hr flow-through and static aquatic toxicity tests have been conducted for many years with cross validation by many laboratories. Some methods were written by Standard Methods and ASTM.

Applications: Water.

Samples: Pure Chemicals: Insecticides, Herbicides, Heavy Metals, PCB's. Complex Mixtures: Industrial - effluents; Energy Related - drilling muds, oils.

Duration: Test: 48 hours; Analysis: 48 hours.

Cost: Static test: $300; Flow-through test: $650.

Interpretation: From the tests the lethal effects of a toxicant along with a statistically valid 95% confidence interval can be determined.

Level of Complexity: I.

OHEE Laboratory Involved: ERL-DUL, Technical Assistance Branch.

Persons to Contact: C. Stephan, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804 (FTS 783-9510, Commercial 218 727-6692 X510).

Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, Inc., Wareham, MA, S. Sauter and K.J. Macek.

Program Office Support: OHEE; ORD.

References: 1) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. EPA-600/3-75-009, U.S. EPA, 1975.

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*This test is also applied to freshwater invertebrates. See 2221.

2322 ACUTE TOXICITY TEST, BENTHIC ASSEMBLAGES

Biological Activity Detected: Toxicity.

Principle: The acute effects of toxic substances on a macrofunal genthic microcosm representative of natural benthic assemblages in the Pacific Northwest will be examined.

Endpoints: Qualitative: Behavior, observe animals able to bury in dredge sediment. Quantitative: Count of survivors and count of animals able to bury in substrate in relation to water concentration provides EC values.

Strengths: Test organism is a sensitive amphipod species, *Paraphoxus epistomus*.

Weaknesses: New species not frequently used in aquatic testing.

Status of Development: Developmental.

Describe: This test procedure has only been conducted at the Newport, Oregon, facility.

Applications: Water; Soil.

Samples: Pure Chemicals: Heavy metals, i.e., Ca, Zn, Cr.

Complex Mixtures: Dredge sediment; Complex wastes.

Duration: Test: 96 hours.

Cost: Not yet determined.

Interpretaton: From this test one can determine if a dredge soil is acutely toxic to estuarine benthic animals.

Level of Complexity: I.

OHEE Laboratory Involved: ERL-COR, Ecological Effects Research Division, Marine and Freshwater Branch, Newport Field Station.

Persons to Contact: R. Swartz, U.S. EPA, ERL-COR, Newport Field Station, Marine Science Center, Newport, OR 97365, (FTS 423-4111, Commercial 503 867-4041).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OTS.

References: 1) U.S. EPA, Corps of Engineers. Appendix F. Guidance for Performing Solid State Bioassays. In: Report on Ecological Evaluation of Proposed Discharge of Dredged Material on Ocean Waters. Tech. Committees on Criteria for Dredge and Fill Material. U.S. Army Waterways Station, Vicksburg, MS, July 1977.

2323 CHRONIC INVERTEBRATE TOXICITY TEST, ESTUARINE SHRIMP (PALAEMONETES PUGIO)

Biological Activity Detected: Toxicity; Growth; Reproduction.
Principle: Determine chronic effects of a pollutant on the entire life cycle of Grass Shrimp, *Palaemonetes pugio*.
Endpoints: *Qualitative*: N/A. *Quantitative*: LC50 values; Significant differences in survival, growth, and fecundity in experimental animals compared to controls.
Strengths: LC50 values; Significant differences in survival; Determination of growth and reproduction. Data give good indication of the effects of a toxicant over the animal's entire life cycle.
Weaknesses: Duration is long, about 5 months for chronic test; 2 to 3 months for partial-chronic.
Status of Development: Developmental.
Describe: Chronic tests have only been conducted at ERL-GB.
Applications: Water.
Samples: *Pure Chemicals*: Pesticides. *Complex Mixtures*: N/A.
Duration: Test: Approximately 5 months.
Cost: $25,000, including analytical back-up.
Interpretation: A maximum acceptable toxicant concentration can be established for this estuarine invertebrate.
Level of Complexity: 3.
OHEE Laboratory Involved: ERL-GB, Experimental Environments Branch.
Persons to Contact: *D.B. Tyler-Schroeder*, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OHEE.
References: 1) Tyler-Schroeder, D.B. Use of the Grass Shrimp, *Palaemonetes pugio*, in a Life Cycle Toxicity Test. Symposium on Aquatic Toxicology and Hazard Evaluation. ASTM, 1978. In press. 2) Bioassay Procedures for the Ocean Disposal Permit Program. EPA-600/9-78-010, U.S. EPA, 1978.

2324 CHRONIC INVERTEBRATE TOXICITY TEST, ESTUARINE MYSID (MYSIDOPSIS BAHIA)

Biological Activity Detected: Toxicity; Fecundity; Growth.
Principal: Determine the chronic effects of toxicants on the entire life cycle of the crustacean species.
Endpoints: Qualitative: N/A. Quantitative: LC50 values; Significant differences in growth, reproduction, and survival.
Strengths: An estimate of chronic toxicity can be determined.
Weaknesses: Relatively high cost and duration of test; Animals are not always available throughout the year.
Status of Development: Being implemented.
Describe: ERL-GB and Bionomics EG & G, a private laboratory, have conducted these tests.
Applications: Water.
Samples: Pure Chemicals: Pesticides; Metals. Complex Mixtures: N/A.
Duration: Test: Approximately 28 days.
Cost: $7,000, including analytical back-up on pure chemicals.
Interpretation: A maximum acceptable toxicant concentration (MATC) can be established for a marine/estuarine invertebrate in this test.
Level of Complexity: 3.
OHEE Laboratory Involved: ERL-GB, Experimental Environments Branch.
Persons to Contact: D.W. Nimmo, U.S. EPA, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).
Grant/Contract Laboratory Involved and Principal Investigators: Bionomics EG & G, Rt. 6, Box 1002, Pensacola, FL 32507, P.R. Parrish.
Program Office Support: OHEE; OWP.
References: 1) Nimmo, D.W., L.H. Bhaner, R.A. Rigby, J.M. Sheppard, and A.J. Wilson. *Mysidopsis bahia*: An Estuarine Species Suitable for Life-Cycle Toxicity Tests to Determine the Effects of a Pollutant. In: Aquatic Toxicology and Hazard Evaluation, Mayer, Hamelink, eds. ASTM, STP 634:109-116, 1977.

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2331 ESTUARINE MICROCOSMY I

Biological Activity Detected: Fate.

Principle: This test assesses transport and degradation potential of aquatic pollutants.

Endpoints: Qualitative: Types of transport; Localization; Mechanisms of degradation and character of degradation products. Quantitative: Rates of above processes; Effect of environmental parameters on rates; EC values.

Strengths: Use of environmental substrates; Mass balance analysis with radiolabeled pollutants; Versatility; Short turn-around time.

Weaknesses: Sealing factors from laboratory systems to the environment.

Status of Development: Developmental.

Describe: Systems have been designed and are in operation. Fate of pesticides has been tested. Optimization of systems are now in progress. Field validation is being initiated.

Applications: Water.

Samples: Pure Chemicals: Pesticides, Toxic Organics. Complex Mixtures: Industrial - effluents; Energy Related - oil.

Duration: Test: 4 to 8 weeks/chemical pollutant.

Cost: $2,000/month, not including senior investigator time.

Interpretation: This test supplies data on transport and degradation in natural system.

Level of Complexity: 2.

OHEE Laboratory Involved: ERL-GB, Processes and Effect Branch.

Persons to Contact: A.W. Bourquin, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011); R.L. Garnas, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011); P.H. Pritchard, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE; OPP; OTS; OEMI.

References: 1) Bourquin, A.W., R.L. Garnas, P.H. Pritchard, F.G. Wilkes, C.R. Cripe, and N.I. Rubinstein. Interdependent Microcosms for the Assessment of Pollutants in the Marine Environment. Internat. J. of Envir. Studies., 1978. In press.

2332 ESTUARINE MICROCOSMY II

Biological Activity Detected: Toxicity.
Principle: This test assesses the toxicity of pollutants to microbial growth and microbial degradation processes.
Endpoints: Qualitative: Types of microorganisms affected; Total biomass reduction; Selection of species; Physiological indies affected; Mechanisms of toxicity. Quantitative: EC values.
Strengths: Quick screen which uses natural assemblages of microorganisms.
Weaknesses: Extrapolation from laboratory systems to the environment; Requires analytical supports.
Status of Development: Being implemented.
Describe: Pesticides and toxic organics have been tested.
Applications: Water.
Samples: Pure Chemicals: Pesticides, Toxic organics, Heavy metals. Complex Mixtures: Industrial - effluents; Energy Related - oil.
Duration: Test: 4 to 8 weeks/chemical pollutant.
Cost: $1,500/month, not including senior investigator time.
Interpretation: This test determines toxicant effects on microbial assemblages.
Level of Complexity: 2.
OHEE Laboratory Involved: ERL-GB, Processes and Effect Branch.
Persons to Contact: A.W. Bourquin, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011); R.L. Garnas, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011); P.H. Pritchard, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).
Grant/Contract Laboratory Involved and Principal Investigators: Georgia State U., Atlanta, GA 30303, D.G. Ahearn; Gulf Coast Research Laboratory, P.O. Box 26518, New Orleans, LA, W. W. Walker.
Program Office Support: OEMI.
References: 1) Bourquin, A.W., P.H. Pritchard, and W.R. Mahaffey. Effects of Kepone on Estuarine Microorganisms. Developments in Industrial Microbiology. 1978. In press. 2) Bourquin, A.W. Effects of Malathion on Microorganisms of an Artificial Salt-Marsh Environment. J. Envir. Quality, 6:383-378, 1977.

2333 ESTUARINE COMMUNITIES

Biological Activity Detected: Toxicity.

Principle: This test determines concentrations of a toxicant which offset the settling and development of benthic estuarine communities. These communities develop in sand substrate from larvae in unfiltered seawater.

Endpoints: Qualitative: N/A. Quantitative: Measure of species diversity; Number of sensitive phyla, biomass, growth, and total number of species; EC values.

Strengths: From this test we can see how a toxicant changes the community makeup by limiting sensitive groups and promoting growth of others.

Weaknesses: Duration is long (2 to 4 months); The investigator must have a good taxonomic background.

Status of Development: Developmental.

Describe: Only ERL-GB and one contractor have completed this type of community study.

Applications: Water.

Samples: Pure Chemicals: Pesticides. Complex Mixtures: Energy Related - drilling muds.

Duration: Test: 2 to 4 months to conduct test; Analysis: another 2 to 4 months to identify the animals.

Cost: $6,000.

Interpretation: From these studies we are able to predict the concentration of a toxicant that will adversely affect the recruitment and development of estuarine benthic communities.

Level of Complexity: 3 to 4.

OHEE Laboratory Involved: ERL-GB, Experimental Environments Branch.

Persons to Contact: M. Tagatz, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).

Grant/Contract Laboratory Involved and Principal Investigators: Florida State U., Tallahassee, FL 32306, B. Glasson.

Program Office Support: OHEE; ORD.

References: 1) Hansen, D.J. Aroclor 1254: Effect on Composition of Developing Estuarine Animal Communities in the Laboratory. Marine Sci., 18:19-33, 1974.

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2411 STRESS ETHYLENE BIOASSAY IN PLANTS

Biological Activity Detected: Alteration of physiological process.

Principle: Environmental stresses cause plants to produce large amounts of ethylene. The elevated ethylene occurs before and often in the absence of visual injury.

Endpoints: Qualitative: Observation of increase of ethylene production; In some cases modification of plant growth form.
Quantitative: Extent of increase for comparison with reference plants and chemicals.

Strengths: More sensitive than visual assessment; Less subject to variability.

Weaknesses: Short duration of the phenomena (approx. 48 hours); The test is designed as a flow-through test and not as a static system.

Status of Development: Being implemented.
Describe: The test has been used to examine effects of ozone and chlorine on a variety of plants ranging from pine trees to potatoes.

Applications: Air.

Samples: Pure Chemicals: Gaseous pollutants: Ozone, SO_2, NO_x, CO. Complex Mixtures: Ambient - air; Industrial - air pollutants; Energy Related - air pollutants; Transportation Related - air pollutants; Other - acid rain.

Duration: Test: 3 to 5 days exclusive of plant rearing; Analysis: Simultaneous with test.

Cost: $2,500.

Interpretation: This test provides evidence of tissue injury. Excess ethylene production may result in defoliation.

Level of Complexity: I.

OHEE Laboratory Involved: ERL-COR, Ecological Effects Research Division, Terrestrial Ecology Branch.

Persons to Contact: D.T. Tingey, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4621).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Tingey, D.T., C. Standley, and R.W. Field. Stress Ethylene Evolution, a Measure of Ozone Effects on Plants. Atmos. Envir., 10:969-974, 1976.

2412 MEASUREMENT OF NITROGENASE ACTIVITY BY ACETYLENE REDUCTION IN NODULATED PLANTS

Biological Activity Detected: Alteration of physiological process.
Principle: Measure reduction of acetylene to ethylene.
Endpoints: Qualitative: N/A. Quantitative: Amount of ethylene produced in relation to pollutant concentration.
Strengths: Requires minimal facilities or equipment; Rapid analysis.
Weaknesses: Needs further evaluation.
Status of Development: Being implemented.
Describe: This test is used to assess the impact of heavy metals (cadmium) on soybean, alder, and alfalfa nitrogen fixation systems.
Applications: Soil.
Samples: Pure Chemicals: Heavy metals. Complex Mixtures: Industrial - sludge.
Duration: Test: 10 to 15 days exclusive of time to grow plants; Analysis; Immediately following test.
Cost: $5,000/chemical.
Interpretation: This test provides information on the ability of nodulated plants to fix nitrogen in the presence of stress.
Level of Complexity: 1.
OHEE Laboratory Involved: ERL-COR, Ecological Effects Research Division, Terrestrial Ecology Branch.
Persons to Contact: C. Wickliff, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4622).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OHEE.
References: 1) Wickliff, C. PhD Thesis, Oregon State U. 1977. 2) Fishbeck, K., H.J. Evans, and L.L. Boersma. Agronomy J., 65:429-433, 1973. 3) Huang, Chi-Ying, F.A. Bazzaz, and L.N. Vanderhoff. Plant Physiology, 54:122-124, 1974.

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2421 TERRESTRIAL MICROCOSM CHAMBER

Biological Activity Detected: Toxicity; Bioaccumulation; Biomagnification; Community processes.

Principle: Radiolabeled pesticides at or below accepted field application rates are applied to assess fate, species and population effects.

Endpoints: Qualitative: Microcosms should be viewed as a tool which at present provide only trends in fate or effects. Quantitative: N/A.

Strengths: Higher link between bench and field; Lower cost than field studies; Provides indices of distribution.

Weaknesses: Cost/unit; Ambiguity of results; Not validated.

Status of Development: Developmental.

Describe: At present ERL-COR is developing a testing protocol consisting of not one system, but a methodology that utilizes "benchmark" data as well as various microcosms depending on the information required.

Applications: Air; Water; Soil.

Samples: Pure Chemicals: Heavy metals, Pesticides, Gaseous pollutants. Complex Mixtures: Industrial - effluents; Energy Related - air pollutants; Transportation Related - air pollutants.

Duration: Test: 2 months; Analysis: 3 months.

Cost: $50 to $100,000, depending on the compound.

Interpretation: Fate results utilized ecological mag: bioaccumulation, biodegradability and degradation. Effects' results are still open to discussion other than acute toxicity.

Level of Complexity: 3.

OHEE Laboratory Involved: ERL-COR, Ecological Effects Research Division, Terrestrial Ecology Branch.

Persons to Contact: J.D. Gile, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4649); J.W. Gillett, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4622).

Grant/Contract Laboratory Involved and Principal Investigators: U. of Michigan, Ann Arbor, MI 48104, E. Goodman; U. of Wisconsin, Madison, Wisconsin 53706, P. Lichtenstein.

Program Office Support: OHEE; OPP.

References: 1) Gillett, J.W., and J.D. Gile. Pesticide Fate in Terrestrial Laboratory Ecosystems. Intern. J. Envir. Studies, 10:15-22, 1976.

2422 SOIL CORE MICROCOSM

Biological Activity Detected: Monitor community processes.

Principle: The use of an intact system provides a more realistic representation of a natural system. The test relies on the production of CO_2 and nutrient loss as indicators of community fitness.

Endpoints: Qualitative: N/A. Quantitative: Rate of CO_2 production; Loss of nutrients through leachate.

Strengths: Small size; Low cost/unit; Simple design.

Weaknesses: High degree of variability between units, due to natural variability.

Status of Development: Developmental.

Describe: To date, only inorganics have been examined in the soil core. ERL-COR is currently evaluating the system for use with a broad spectrum of toxics.

Applications: Soil.

Samples: Pure Chemicals: Heavy metals, Pesticides. Complex Mixtures: Industrial - waste effluents.

Duration: Test: 3 months; Analysis: 1 to 2 months.

Cost: $2,000 to $3,000.

Interpretation: This test reflects the ability of a community to decompose material. It also reflects the impact of a chemical on nutrient cycling.

Level of Complexity: 3.

OHEE Laboratory Involved: ERL-COR, Ecological Effects Research Division, Terrestrial Ecology Branch.

Persons to Contact: J. Gile, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4649).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OTS.

References: 1) Draggon, S. Soil Core Microcosm. Proceedings of EPA Workshop on Terrestrial Microcosms. J.W. Gillett, ed. Corvallis, OR, 1977. In press.

2423 SOIL/LITTER MICROCOSM

Biological Activity Detected: Monitor decomposition process.

Principle: Carbon dioxide production and O_2 consumption are monitored as indicators of microbial respiration.

Endpoints: Qualitative: O_2; CO_2; Microbiota; Microarthropods; Nematodes. Quantitative: Change in respiratory rate with changing chemical concentration.

Strengths: Low cost/unit; Simple analytical technique and equipment; Overall simplicity of system.

Weaknesses: Limited type of data generated.

Status of Development: Developmental.

Describe: Soil is tested in 1 qt. mason jars with respiration CO_2 and O_2 measured by simple titrametric or gasometric techniques.

Applications: Soil.

Samples: Pure Chemicals: Heavy metals, Pesticides. Complex Mixtures: Ambient; Industrial; Energy Related.

Duration: Test: 1 to 4 weeks; Analysis: Simultaneous with test.

Cost: $500/chemical.

Interpretation: Changes in respiration may reflect the ability of a community to decompose organic matter.

Level of Complexity: 3.

OHEE Laboratory Involved: ERL-COR, Ecological Effects Research Division, Terrestrial Ecology Branch.

Persons to Contact: B. Lighthart, U.S. EPA, ERL-COR, 200 SW 35th St., Corvallis, OR 97330, (FTS 420-4832).

Grant/Contract Laboratory Involved and Principal Investigators: N/A.

Program Office Support: OHEE.

References: 1) Soil/Litter Microcosm. In: Proceedings of EPA Workshop on Terrestrial Microcosms. J.W. Gillett, ed. U.S. EPA, Corvallis, OR, 1977. In press.

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2501 BIOCONCENTRATION STUDIES, MARINE AND FRESHWATER ANIMALS

Biological Activity Detected: Bioconcentration.
Principle: To determine the uptake and depuration rates of pure chemicals in tissues.
Endpoints: Qualitative: Flesh tainting. Quantitative: Uptake rates; Depuration rates; Bioconcentration factors (uptake of a pure chemical in tissues divided by the concentration of the chemical in the exposure water).
Strengths: A good estimate of the uptake, depuration rates of a pure chemical, plateau level, and bioconcentration factors.
Weaknesses: Duration at least 1 month; Requires complicated chemical analyses; Does not always reflect a true picture of bioconcentration in the field because of uptake from other sources (of food and sediment).
Status of Development: Being implemented.
Describe: Various laboratories within EPA and private industry are now using this method. The procedure has not yet been accepted by ASTM as it is still being revised.
Applications: Water.
Samples: Pure Chemicals: Insecticides, Herbicides, PCB's, Chlorinated hydrocarbons, Radiolabeled material. Complex Mixtures: N/A.
Duration: Test: 30 to 60 days; Analysis: 30 to 60 days.
Cost: $4,000.
Interpretation: From the above tests the potential of contaminants reaching man's seafoods can be determined.
Level of Complexity: 2.
OHEE Laboratory Involved: ERL-DUL, Research Branch, Chemical Pollutant Section; ERL-GB, Experimental Environments Branch.
Persons to Contact: G.D. Veith, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9534); S.C. Schimmel, ERL-GB, Sabine Island, Gulf Breeze, FL 32561, (FTS 686-9011).
Grant/Contract Laboratory Involved and Principal Investigators: Bionomics, EG & G, Rt. 6, Box 1002, Pensacola, FL 32507, P.R. Parrish.
Program Office Support: OHEE; OPP.
References: 1) ASTM Committee on Bioconcentration of Test Materials in Fishes and Oysters. (E-35 Committee on Pesticides). In preparation.

2502 REVERSE-PHASE HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

Biological Activity Detected: Bioconcentration.
Principle: HPLC retention time correlates with the logarithm of the partition coefficient which correlates with the bioconcentration factor of organic chemicals in fish tissue.
Endpoints: Qualitative: N/A. Quantitative: Provides bioconcentration potential of organic chemicals in animal tissue.
Strengths: Rapid; Inexpensive.
Weaknesses: Requires a HPLC.
Status of Development: Being implemented.
Describe: Chemical analysis is utilizing HPLC to obtain Log P values, which can be correlated with water solubility and bioconcentration factors for organic chemicals.
Applications: Water.
Samples: Pure Chemicals: Organic; Inorganic. Complex Mixtures: Industrial.
Duration: Test: 10 to 20 min; Analysis: 10 to 20 min.
Cost: $100.
Interpretation: This work sets forth a rapid, inexpensive method for screening chemicals for their bioconcentration potential in the environment.
Level of Complexity: 0.
OHEE Laboratory Involved: ERL-DUL, Research Branch, Physiological Effects of Pollutants Section.
Persons to Contact: G.D. Veith, U.S. EPA, ERL-DUL, 6201 Congdon Blvd., Duluth, MN 55804, (FTS 783-9534, Commercial 218 727-6692 X534).
Grant/Contract Laboratory Involved and Principal Investigators: N/A.
Program Office Support: OHEE; ORD.
References: 1) Veith, G.D., and Morris. Ecol. Rept. Series, U.S. EPA, 1978. In press. 2) Lee, Huges, and G.D. Veith. Water, Air, and Soil Pollution, 8:749-484, 1977. 3) Veith, G.D., and N.M. Austin. Detection and Isolation of Bioaccumulable Chemicals in Complex Effluents. In: Identification and Analysis of Organic Pollutants in Water. L.A. Keith, ed. Ann Arbor Science Publishers, Ann Arbor, MI, 1976. pp. 297-304.

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*Integrated Systems are listed twice: once under "Integrated Systems" and once under the title proper.

†This test is listed under two titles.

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Fruit Flies		12110	
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*An underlined number indicates that the test system is being applied to more than one organism/species/test type.

INDEX III: EXPERIMENTAL SUBJECT LISTING (continued)

ORGANISM — SPECIES	TEST TYPE		
	IN-VITRO	IN-VIVO	OTHER
		TEST SYSTEM NUMBER	
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		TEST SYSTEM NUMBER	
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Anderson, R.L.	ERL-DUL	2222, 2223.
Bercz, J.P.	HERL-CIN	1131, 1211, 1213, 1236.
Biesinger, K.E.	ERL-DUL	2221.
Bourquin, A.W.	ERL-GB	2331, 2332.
Brown, M.M.	HERL-RTP	1216.
Brungs, W.A.	ERL-DUL	2212, 2213.
Bull, R.J.	HERL-CIN	1113, 1121, 1131, 1132, 1136, 1155, 1156, 1226, 1236, 1306.
Campbell, K.I.	HERL-CIN	1152.
Carlson, R.	ERL-DUL	2214.
Casciano, D.	NCTR	1211, 1217, 1227.
Chadwick, R.W.	HERL-RTP	1135.
Charles, J.	HERL-RTP	1141, 1142.
Chernoff, N.	HERL-RTP	1303, 1304.
Clarke, N.	HERL-CIN	1215.
Claxton, L.	HERL-RTP	1211, 1212, 1214, 12113, 1221, 1222.
Copeland, M.F.	HERL-RTP	1135.
Couch, J.	ERL-GB	1241.
Courtney, K.D.	HERL-RTP	1301, 1302, 1305.
Daniel, B.	HERL-CIN	1215, 1228.
Drummond, R.	ERL-DUL	2214.
Gardner, D.E.	HERL-RTP	1132 1136 1143, 1144, 1149, 11410.

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*An underlined number indicates that the test system is used by more than one of the above indicators.

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Garnas, R.L.	ERL-GB	2331, 2332.
Gentile, J.	ERL-NAR	2102.
Gile, J.D.	ERL-COR	2421, 2422.
Gillett, J.W.	ERL-COR	2421.
Graham, J.A.	HERL-RTP	1114, 1117.
Green, J.C.	ERL-COR	2101.
Hansen, D.J.	ERL-GB	2312, 2313.
Hatch, G.	HERL-RTP	1115, 1137, 1138.
Hedtke, S.F.	ERL-DUL, NFTS	2241.
Huisingh, J.L.	HERL-RTP	1119, 11110, 11111, 11112, 11113, 1211, 1212, 1221.
Jackim, E.	ERL-NAR	1229.
Kavlock, R.	HERL-RTP	1303, 1304.
Kowal, N.E.	HERL-CIN	1119, 1219, 1236.
Lazear, E.	NCTR	1211.
Lee, S.D.	HERL-CIN	1111, 1112.
Lighthart, B.	ERL-COR	2423.
Linder, R.	HERL-RTP	1122.
Lingg, R.D.	HERL-CIN	1133, 1134.
Malcolm, A.R.	ERL-NAR	1218, 1225, 1229.
McCabe, L.J.	HERL-CIN	1211, 12111.
McKim, J.M.	ERL-DUL	2212.
Miller, W.E.	ERL-COR	2101.
Moore, W.	HERL-CIN	1143, 1144.
Nesnow, S.	HERL-RTP	1134, 1231, 1232, 1234, 1235.
Nimmo, D.W.	ERL-GB	2324.
O'Neil, J.J.	HERL-RTP	1145.
Orthoefer, J.	HERL-CIN	1237.
Pahren, H.	HERL-CIN	1116, 1118, 1211, 1213, 1233.

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Pesch, G.G.	ERL-NAR	1225.
Pritchard, P.H.	ERL-GB	2331, 2332.
Reiter, L.	HERL-RTP	1151, 1153, 1154.
Richards, N.	ERL-GB	1211, 1242, 1243, 1244, 1245, 1246.
Robinson, E.	ERL-DUL, NFTS	2101.
Sandhu, S.S.	HERL-RTP	1212, 1213, 1214, 1216, 1217, 1219, 12110, 12111, 12112, 12113, 1221, 1223, 1225.
Schimmel, S.C.	ERL-GB	2501.
Schoor, P.	ERL-GB	1242.
Shiroyama, T.	ERL-COR	2101.
Specht, D.	ERL-COR	2102.
Spehar, R.	ERL-DUL	2213.
Spoor, W.A.	ERL-GB	2215.
Stara, J.F.	HERL-CIN	1211, 1214, 12113.
Stephan, C.	ERL-DUL	2211, 2221, 2224, 2311, 2321.
Swartz, R.	ERL-COR	2322.
Tagatz, M.	ERL-GB	2333.
Tingey, D.T.	ERL-COR	2411.
Tyler-Schroeder, D.B.	ERL-GB	2323.
Veith, G.D.	ERL-DUL	2501, 2502.
Walsch, G.	ERL-GB	2102.
Waters, M.D.	HERL-RTP	1119, 11111, 1211, 1212, 1213, 1214, 1216, 1217, 1219, 12110, 12111, 12112, 12113, 1221, 1222, 1223, 1224, 1225, 1234.
Wickliff, C.	ERL-COR	2412.
Wiester, M.J.	HERL-CIN	1147.

INDEX V: U.S. EPA LABORATORIES IN ALPHABETICAL ORDER

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Environmental Research Laboratory-Corvallis, Oregon	2101, 2102, 2322, 2411, 2412, 2421, 2422, 2423.
Environmental Research Laboratory-Duluth, Minnesota	2101, 2211, 2212, 2213, 2214, 2215, 2221, 2222, 2223, 2224, 2231, 2241, 2311, 2321, 2501, 2502.
Environmental Research Laboratory-Gulf Breeze, Florida	1211, 1241, 1242, 1243, 1244, 1245, 1246, 2102, 2312, 2313, 2323, 2324, 2331, 2332, 2333, 2501.
Environmental Research Laboratory-Narragansett, Rhode Island	1218, 1225, 1229, 2102.
Health Effects Research Laboratory-Cincinnati, Ohio	1111, 1112, 1113, 1116, 1118, 1119, 1121, 1131, 1132, 1133, 1134, 1136, 1143, 1144, 1146, 1147, 1148, 1152, 1155, 1156, 1211, 1213, 1214, 1215, 1219, 12111, 12113, 1226, 1228, 1233, 1236, 1238, 1306.
Health Effects Research Laboratory-Research Triangle Park, North Carolina	1114, 1115, 1117, 1119, 11110, 11111, 11112, 11113, 1122, 1132, 1134, 1135, 1136 1137, 1138, 1141, 1142, 1143, 1144, 1145, 1149, 11410, 1151, 1153, 1154, 1211, 1212, 1213, 1214, 1216, 1217, 1219, 12110, 12111, 12112, 12113, 1221, 1222, 1223, 1224, 1225 1227, 1231, 1232, 1234, 1235, 1301, 1302, 1303, 1304, 1304.
National Center for Toxicological Research-Jefferson, Arkansas	1211, 1217, 1227.

*An underlined number indicates that the test system is used by more than one laboratory.

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DEPARTMENT	U.S. EPA LABORATORY	CONTACT PERSONNEL
	ENVIRONMENTAL RESEARCH LABORATORY-CORVALLIS 200 SW 35th Street Corvallis, Oregon 97330	
Assessment Criteria Division		
Special Studies Branch		J.C. Green W.E. Miller T. Shiroyama
Ecological Effects Research Division		
Marine and Freshwater Branch		D. Specht
Newport Field Station		R. Swartz
Terrestrial Ecology Branch		S. Draggon J.D. Gile J.W. Gillett B. Lighthart D.T. Tingey C. Wickliff
	ENVIRONMENTAL RESEARCH LABORATORY-DULUTH 6201 Congdon Boulevard Duluth, Minnesota 55803	
Extramural Program Branch		K.E. Biesinger
Newtown Fish Toxicology Station		S.F. Hedtke E. Robinson
Research Branch		
Chemical Pollutant Section		G.D. Veith
Physical Pollutant Section		R.L. Anderson
Physical Effects of Toxicants Section		W.A. Brungs R. Carlson R. Drummond J.M. McKim R. Spehar

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Technical Assistance Branch		C. Stephan
	ENVIRONMENTAL RESEARCH LABORATORY-GULF BREEZE Sabine Island Gulf Breeze, Florida 32561	
Experimental Environments Branch		D.J. Hansen D.W. Nimmo S.C. Schimmel M. Tagatz D.B. Tyler-Schroeder C. Walsch
Office of the Director (Carcinogenic Research Team)		J. Couch N. Richards W.A. Spoor
Processes and Effects Branch		A.W. Bourquin R.L. Garnas P.H. Pritchard
	ENVIRONMENTAL RESEARCH LABORATORY-NARRANGANSETT South Ferry Road Narragansett, Rhode Island 02882	
Toxicology Branch Genetic Toxicology Team		E. Jackim A.R. Malcolm G.G. Pesch
Marine Toxicology Team		J. Gentile
	HEALTH EFFECTS RESEARCH LABORATORY-CINCINNATI 26 West St. Clair Street Cincinnati, Ohio 45268	
Field Studies Division Toxicological Assessment Branch		J.P. Bercz N.E. Kowal L.J. McCabe H. Pahren J.F. Stara

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Laboratory Studies Division		
Exposure Evaluation Branch		R.D. Lingg
Functional Pathology Branch		S.D. Lee J. Orthoefer W. Moore W.E. Pepelko M.J. Wiester
Toxicological Assessment Branch		R.J. Bull K.I. Campbell N. Clarke B. Daniel
	HEALTH EFFECTS RESEARCH LABORATORY-RESEARCH TRIANGLE PARK Research Triangle Park North Carolina 27711	
Clinical Studies Division		
Biomedical Research Branch		D.E. Gardner J.A. Graham G. Hatch J.J. O'Neil
Experimental Biology Division		
Developmental Biology Branch		N. Chernoff R. Kavlock
Neurobiology Branch		L. Reiter
Environmental Toxicology Division		
Biochemistry Branch		M.M. Brown R.W. Chadwick L. Claxton M.F. Copeland J.L. Huisingh S. Nesnow S.S. Sandhu M.D. Waters

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	HEALTH EFFECTS RESEARCH LABORATORY-RESEARCH TRIANGLE PARK (continued)	
Toxic Effects Branch		J. Charles K.D. Courtney R. Linder
	NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH Jefferson, Arkansas 72079	
Division of Mutagenesis Research Somatic Cell Section		D.A. Casciano E. Lazear

INDEX VII: GRANT/CONTRACT LABORATORIES IN ALPHABETICAL ORDER

GRANT/CONTRACT LABORATORY	PRINCIPAL INVESTIGATOR	TEST SYSTEM NUMBER
American Health Foundation Naylor Dana Institute for Disease Prevention Hammond House Road Valhalla, New York 10595	G.M. Williams	1227.
Ball State University Muncie, Indiana 47306	D. Adalis	1114.
Bionomics EG & G Route 6, Box 1002 Pensacola, Florida 32507	P.R. Parrish	2501, 2312, 2313, 2324.
Bionomics, Inc. Wareham, Massachusetts	K.S. Macek	2211, 2212, 2221, 2311, 2321.
	S. Sauter	2211, 2212, 2221 2311, 2321.
Brookhaven National Laboratories Long Island, New York	L. Shirer	12111.
California, University of Davis, California 95616	E. Goldstein	11410.
California, University of School of Medicine Los Angeles, California 90032	M.G. Mustafa	1112.
California, University of Medical Center San Francisco, California 94132	R.S. Bhatnagar	1111.
Cincinnati, University of Cincinnati, Ohio 45221	C. Smith	1133.
Cincinnati University of Medical Center Cincinnati, Ohio 43221	J.C. Loper	1211, 1236.
	D. Lang	1236.

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GRANT/CONTRACT LABORATORY	PRINCIPAL INVESTIGATOR	TEST SYSTEM NUMBER
Colorado, University of Medical Center 4200 East 9th Avenue, Denver Colorado 80262	C.C. Solomons W.L. Weston	1116. 1118.
Denver Research Institute Denver, Colorado 80210	J. Schmidt-Coderis	1246.
Florida State University Tallahassee, Florida 32306	B. Glasson	2333.
Georgia State University Atlanta, Georgia 30303	D.G. Ahearn	2332.
Gulf Coast Research Laboratory P.O. Box Drawer AG Ocean Springs, Mississippi 39564	W.W. Walker	2332.
Gulf South Research Institute P.O. Box 26518 New Orleans, Louisiana	N. Gruener E. Kline	1119, 1219, 1236. 1245.
IIT Research Institute 10 West 35th Street Chicago, Illinois 60616	C. Aranyi R. Ehrlich L. Schiff	11111, 11410. 1149. 1114.
Illinois, University of Urbana, Illinois 61801	M. Plewa	12112.
Iowa State University Ames, Iowa 50010	W.E. Lloyde	1153, 1154.
Litton Biometics, Inc. Nicholson Lane Kensington, Maryland	D.T. Brusick	1211.
Louisiana State University Medical School New Orleans, Louisiana 70112	W. Pelon	1211.
Medical College of Virginia Richmond, Virginia 23298	R.L. Balster J.F. Borzelleca W.L. Dewey A.E. Munson	1152. 1152. 1152. 1152.

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Michigan, University of Ann Anbor, Michigan 48104	A. Beaudoin	1305.
	E. Goodman	2421.
Microbiological Associates Bethesda, Maryland 20014	R. Kouri	1231, 1235, 1237.
	L. Schectman	1231, 1235, 1237.
Missouri, University of Columbia, Missouri 65201	C. Marianseld	1211.
	J.T. O'Conner	12111.
North Carolina, University of Chapel Hill, North Carolina 27514	A. Collier	1114.
	D. Humm	1244.
Northrop Services, Inc. P.O. Box 12313 Research Triangle Park, North Carolina 27709	B. Adkins	1114, 1149.
	N.E. Garrett	1119, 11110, 11111, 11113.
Oak Ridge National Laboratory P.O. Box Y Oak Ridge, Tennessee 37830	A. Hsie	1217.
Ohio State University Columbus, Ohio 43210	R.W. Hart	1226.
Oregon, University of Eugene, Oregon 97403	M. Mix	1241.
Rockefeller University 1230 York Avenue New York, New York 10021	M. Bowers	1117.
Southern Mississippi, University of Hattiesburg, Mississippi 39401	B.J. Martin	1241.
Southwest Research Institute San Antonio, Texas 78284	E. Gause	11410.
Stanford Research Institute Menlo Park, California 94025	A. Mitchell	1216, 1219, 12110, 1224.
	G. Newell	12111, 12113.
	V.F. Simmons	1211, 1212, 1213, 1214, 1221, 1222, 1223, 1225.

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Syracuse Research Corporation Merrill Lane Syracuse, New York 13210	J. Saxena	1233.
University of Texas Medical Branch Galveston, Texas 77550	M. Legator	1211, 1214, 12113.
University of West Florida Pensacola, Florida 32504	J. Bazlis	1211.
	R. Rao	1242, 1243, 1245.
Wisconsin, University of Madison, Wisconsin 53706	P. Lichtenstein	2421.

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Motor and sensory activity	1151, 1152, 1153, 1154.

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INDEX IX: ENDPOINTS OF THE GENERAL AND PERINATAL TOXICITY TEST SYSTEMS (continued)

ENDPOINTS	TEST SYSTEM NUMBER
Mutation frequency	1113.
NADH	1113, 1156.
NADPH	1113, 1156.
Neutrophil phagocytosis	1118.
Oxidatant production in alveolar macrophages	1137.
Phagocytic index	1118. 11111.
Platelet function	1115, 1116.
Post natal development	1302, 1304, 1305, 1306.
Protein determinations	1119, 1305.
Pulmonary mechanics	1145, 1146, 1147.
Residual lung volumes	1145, 1146, 1147.
Righting reflex	1132.
Sensory modality	1151, 1152, 1153.
Sequencing of behavior	1151, 1152, 1153.
Serum constituents	1131.
Serum isoenzyme patterns	1131, 1302.
Sleep-time	1132.
Somite development	1305.
Static compliance curves	1145, 1146, 1147.
Task discrimination	1151, 1154.
Teratogenicity	1301, 1302, 1303, 1304, 1305, 1306.
Thyroid function	1131.

(continued)

INDEX IX: ENDPOINTS OF THE GENERAL AND PERINATAL TOXICITY TEST SYSTEMS (continued)

ENDPOINTS	TEST SYSTEM NUMBER
Tidal volume	1145, 1147.
Total cell protein	1119, 11111, 11112.
Urinary constituents	1131.
Viability of neonatal rats	1304, 1306.

INDEX X: ENDPOINT DISTRIBUTION OF THE GENOTOXICITY TEST SYSTEMS

ENDPOINTS	TEST TYPE		
	IN-VITRO	IN-VIVO	OTHER
	TEST SYSTEM NUMBER*		
Carcinogenesis	1231 1232 1233 1234 1235 1236	1237 1241 1242 1246	
Mutagenesis			
Chromosomal aberrations	1225	12113 1225	1218 1219 12110
Point mutation	1211 1212 1214 1216 1217 1218 1219	1213 1214 12110 12111 12112 1242 1245	
Primary DNA Damage	1215 1218 1219 1221 1222 1224 1227 1229	1223 1226 1228 1242	1245

*An underlined number indicates that the test system has more than one endpoint.

INDEX XI: ENDPOINT DISTRIBUTION OF THE ECOLOGICAL TEST SYSTEMS

ENDPOINT	TEST SYSTEM NUMBER*
Bioconcentration Factors (BCF)	2501, 2502.
Effect Concentration (EC)	2101, 2102, 2212, 2214, 2215, 2223, 2224, 2231, 2312, 2313, 2322, 2331, 2332, 2333, 2411, 2412, 2421, 2422, 2423.
50% Effect Concentration (EC50)	2221, 2222, 2321.
50% Lethal Concentration (LC50)	2211, 2221, 2222, 2223, 2224, 2311, 2312, 2321, 2323, 2324.
Maximum Acceptable Toxicant Concentration (MATC)	2212, 2213, 2241†, 2312.

*An underlined number indicates that the test system has more than one endpoint.

†This endpoint is based on change within the ecosystem processes.

INDEX XII: APPLICATION DISTRIBUTION TABLE

APPLICATION:	AIR	WATER	SOIL	FOOD	MULTIMEDIA
TEST SYSTEM NUMBER:					1111
					1112
					1113
	1114				
	1115	1115		1115	
		1116			
					1117
		1118			
	1119	1119			
	11110	11110			
	11111	11111			
					11112
	11113	11113			
					1121
					1122
					1131
					1132
					1133
					1134
					1135
					1136
					1137
	1138	1138		1138	1138
	1141				
	1142				
					1143
					1144
	1145				
	1146	1146		1146	
	1147				1147
	1148	1148		1148	
	1149				
	11410				
					1151
		1152			
					1153
					1154
		1155			

(continued)

INDEX XII: APPLICATION DISTRIBUTION TABLE (continued)

APPLICATION:	AIR	WATER	SOIL	FOOD	MULTIMEDIA
TEST SYSTEM NUMBER:					1156
					1211
					1212
					1213
					1214
					1215
					1216
					1217
					1218
					1219
					12110
	12111	12111			
	12112		12112		12112
					12113
					1221
					1222
					1223
					1224
					1225
					1226
					1227
					1228
		1229			1229
	1231				
					1232
		1233			
	1234				
	1235				
		1236			
					1237
		1241			
		1242			
		1243			
		1244			
		1245		1245	
		1246			1246
					1301
					1302
					1303
	1304	1304		1304	
					1305
					1306
		2101			
		2102			
		2211			

(continued)

INDEX XII: APPLICATION DISTRIBUTION TABLE (continued)

APPLICATION:	AIR	WATER	SOIL	FOOD	MULTIMEDIA
TEST SYSTEM NUMBER:		2212			
		2213			
		2214			
		2215			
		2221			
		2222			
		2223			
		2224			
		2231			
		2241			
		2311			
		2312			
		2313			
		2321			
		2322	2322		
		2323			
		2324			
		2331			
		2332			
		2333			
	2411				
			2412		
	2421	2421	2421		
			2422		
			2423		
		2501			
		2502			

INDEX XIII: SAMPLE DISTRIBUTION OF THE GENERAL AND PERINATAL TEST SYSTEMS

SAMPLES	TEST SYSTEM NUMBER
Aerosols	1141.
Air	1113, 1114, 1115, 1141, 1142, 1145, 1146, 1147, 1148, 1149, 11410.
Alkylating agents	11113.
Aromatic amines	11113.
Asbestos	1146.
AWT effluent	1119.
Benzene	1152.
Cadmium	1111, 1112, 1114, 11111, 1149, 11410.
Chlorinated aliphatic hydrocarbons	1133, 1134.
Chlorinated aromatic hydrocarbons	1133, 1134.
Coal dust	1146, 1148.
Copper	1111.
Diesel fuel	1111, 1113, 1145, 1146, 1147, 1148.
Dioxin	1152, 1301.
Dolomite	11110.
Drugs	1135.

(continued)

INDEX XIII: SAMPLE DISTRIBUTION OF THE GENERAL AND PERINATAL TOXICITY TEST SYSTEMS (continued)

SAMPLES	TEST SYSTEM NUMBER
Energy related pollutants	1113, 1114, 1115, 1117, 11110, 11111, 1132, 1138, 1145, 1146, 1149, 11410, 1155, 1156, 1306.
Ethers	1152.
Fly ash	1119, 11110, 11111.
Gases	1111, 1145, 1149, 11410.
Gasoline exhaust	1113, 1114, 1115, 1146.
Heavy metals	1111, 1119, 11113, 1146, 1151, 1154, 1303, 1306.
Hydrocarbons	1116, 1118. 11113.
Industrial pollutants	1113, 1114, 1115, 1117, 1119, 11110, 11111, 1132, 1138, 1141, 1142, 1146, 1148, 1149, 11410, 1155, 1156, 1306.
Inorganics	1119, 11112, 1301, 1302, 1303, 1304, 1306.
Manganese	1111, 11111, 11410.
Mercury	1111, 1112.
Nickel	1114, 11111, 1149, 11410.
Nitrogen oxides	1111, 1138, 1145.
Nitroso amines	11113.
NO	1138.
NO_2	1111, 1112, 1137, 1138, 1145, 1149, 11410.
Non-ionizing radiation	1151.

(continued)

INDEX XIII: SAMPLE DISTRIBUTION OF THE GENERAL AND PERINATAL TOXICITY TEST SYSTEMS (continued)

SAMPLES	TEST SYSTEM NUMBER
Organics	1116, 1119, 11112, 1121, 1122, 1131, 1145, 1155, 1301, 1302, 1303, 1304, 1305, 1306.
Oxidants	1112, 1146, 1148.
Ozone	1112, 1137, 1138, 1145, 1148, 1149, 11410.
Paraquat	1145, 1148.
Particulates	11110, 1137, 1141, 1145, 1149, 11410.
Pesticides	1122, 1135, 1141, 1142 1151, 1152, 1301, 1302, 1303, 1304, 1305.
Phenyls	1152.
Platium	11111.
Radioactive aerosols	1144.
Rock dust	1148.
Silicic acid	11110.
SO_2	1111, 1112, 1145, 1147, 1148.
Stack gases	1146, 1148.
Sulfates	1145, 1146, 1148, 1149.
Sulfuric acid	1114, 1145.
Sulfur oxides	1111, 1145.
Technical grade materials	1122, 1141, 1142, 1143.

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INDEX XIII: SAMPLE DISTRIBUTION OF THE GENERAL AND PERINATAL TOXICITY TEST SYSTEMS (continued)

SAMPLES	TEST SYSTEM NUMBER
Toxic substances	11110, 1121, 1122, 1131, 1132, 1135, 1141, 1142, 1143, 1145, 1151, 1152, 1301, 1302, 1303, 1304, 1305.
Trace metals	1111, 1119, 11111, 1148, 11410, 1151, 1155, 1303, 1306.
Transportation related pollutants	1113, 1114, 1115, 1117, 1138, 1145, 1146, 1147, 1148, 1149, 11410, 1155, 1156, 1306.
Trihalomethanes	1152.
Vaporizable liquids	1142.
Water	1115, 1116.
Xenobiotics	1135, 1136.
Zinc	1111.

INDEX XIV: SAMPLE DISTRIBUTION OF THE GENOTOXICITY TEST SYSTEMS*†

SAMPLES	TEST SYSTEM NUMBER
ALKYLATING AGENTS	
Aldehydes	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110.
Alkane sulfonic esters	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 12113, 1221, 1222, 1223, 1224.
Alkyl and alkane halides	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223.
Alkylsulfates	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223.
Aryldialkyltriazenes	1212, 12110, 12111.
Aziridines	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 12113, 1221, 1222, 1223.
Azoxy and hydrazo alkanes	1211, 12110, 1224.
Diazoalkanes	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110.
Epoxides	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 12113, 1221, 1222, 1223.
Lactones	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223, 1224,
Nitrogen, sulfur, and oxide mustards	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12113, 1221, 1222, 1223, 1224.
Phosphoric acid esters	1211, 1212, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223.
Sultones	1211, 1212, 1213, 1221, 1222, 1223, 1224.

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*The data base on test systems 1214, 1215, and 12112 is not yet available.
†The scope of this index extends beyond the test system texts.

INDEX XIV: SAMPLE DISTRIBUTION OF THE GENOTOXICITY TEST SYSTEMS (continued)

SAMPLES	TEST SYSTEM NUMBER
ALKYLATING AGENTS (continued)	
Triazines	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 12113, 1224.
AROMATIC AMIDES	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 12113, 1224.
AZO DYES	1211, 1212, 1216, 1217, 1218, 1219, 12110, 1224.
HALOGENATED ETHERS AND HALOHYDRINS	1211, 1212, 1216, 1217, 1218, 1219, 12110, 12111.
HALOGENATED HYDROCARBONS AND RELATED DERIVATIVES	
Fluorocarbons	1211, 1212, 1213, 12110.
Halogenated aromatics	1211, 1212, 12110, 12111.
Vinyl and vinylidine derivatives	1211, 1212, 1216, 1218, 1219, 12110.
HETEROCYCLICS	
Acridines and quinicrines	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223, 1224.
Benzimidazoles	1211, 1212, 12110.
Cyclodienes	1211, 1212.
Dibenzo-p-dioxins	1211, 1212.
Dicarboximides	1211, 1212.
Fluorenones	1211, 1212, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223, 1224.
Fuorocoumarins	1211, 1212.
Phenothiazines	1211, 1212.
Thioxanthines	1211, 1212, 1213, 1216, 1217, 1218, 1219.
Other	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111.

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INDEX XIV: SAMPLE DISTRIBUTION OF THE GENOTOXICITY TEST SYSTEMS (continued).

SAMPLES	TEST SYSTEM NUMBER
HYDRAZINES, HYDROXYLAMINES CARBAMATES, HYDRAZIDES, AND UREAS	
Carbamates	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223.
Hydrazides	1211, 1212, 12111.
Hydrazines	1211, 1212, 12110.
Hydroxylamines	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223.
Ureas and thioureas	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111.
INORGANIC DERIVATIVES	
Halogens and derivatives	1211, 1212, 1216, 1217, 1218, 1219, 12110.
Metal and metalloid derivatives	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223.
Ozone	1212, 12110, 12111.
Sulfur and nitrogen oxides	1211, 1212, 1213, 12110, 12111, 1221, 1222, 1223.
NATURAL PRODUCTS	
Antibotics	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223, 1224.
Mycotoxins	
Aflatoxin	1211, 1212, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223, 1224.
Other	1211, 1212, 12110, 1221, 1222, 1223, 1224.
Pyrrolizidine alkaloids	12110, 1221, 1222, 1223.
Steroids	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110.
Xanthines	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223, 1224.
NITRO DERIVATIVES	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111.

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INDEX XIV: SAMPLE DISTRIBUTION OF THE GENOTOXICITY TEST SYSTEMS (continued)

SAMPLES	TEST SYSTEM NUMBER
NITRO DERIVATIVES (continued)	
Nitrofurans	1211, 1212, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223.
Nitroimidazoles	1212, 12110.
Nitroquinolines and compounds	1211, 1212, 1213, 12110, 1221, 1222, 1223, 1224.
N-NITROSO COMPOUNDS	
Nitrosamides	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223, 1224.
Nitrosamines	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 1221, 1222, 1223, 1224.
Nitrosoureas	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110.
NUCLEIC ACID BASES AND ANALOGS	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110, 12111, 12113.
ORGANIC PEROXIDES	1211, 1212, 12110.
ORGANO-METALLICS	
Organo lead derivatives	1221, 1222, 1223.
Organo mercury derivatives	1211, 1212, 12111, 1221, 1222, 1223.
Other	1211, 1212, 12110, 1221, 1222, 1223.
POLYNUCLEAR AROMATICS	1211, 1212, 1216, 1217, 1218, 1219, 12110, 1221, 1222, 1223, 1224.
OTHER	
Esters and anhydrides	1211, 1212, 1213, 12110, 12111.
N-oxides	1211, 1212, 1213, 12110.
Quaternary ammonium compounds	1211, 1212, 1213, 1216, 1217, 1218, 1219, 12110.
Quinones	1211, 1212, 1216, 1217, 1218, 1219, 12110, 12111.
Sulfites	1212, 1213, 12110, 12111.

INDEX XV: SAMPLE DISTRIBUTION OF THE ECOLOGICAL TEST SYSTEMS

SAMPLES	TEST SYSTEM NUMBER
Acid rain	2411.
Air pollutants	2411, 2421.
Ambient	2101, 2102, 2411, 2423.
Ambient air	2411.
Chloramines	2102.
Chlorinated hydrocarbons	2501.
Chlorine	2102.
CO	2411.
Coal storage	2101.
Complex wastes	2322.
Detergents	2101.
Dissolved gases	2215.
Dredge sediment	2102, 2322.
Drilling muds	2211, 2221, 2311, 2321, 2333.
Energy related	2101, 2211, 2212, 2215, 2221, 2224, 2231, 2311, 2321, 2331, 2332, 2333, 2411, 2423.
Gaseous pollutants	2411, 2421.
Heavy metals	2101, 2102, 2211, 2221, 2222, 2223, 2311, 2321, 2322, 2332, 2412, 2421, 2422, 2423, 2501.

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INDEX XV: SAMPLE DISTRIBUTION OF THE ECOLOGICAL TEST SYSTEMS (continued)

SAMPLES	TEST SYSTEM NUMBER
Herbicides	2101, 2211, 2221, 2311, 2312, 2313. 2321.
Industrial	2101, 2102, 2211, 2212, 2214, 2215, 2221, 2224, 2231, 2311, 2321, 2331, 2332, 2411, 2412, 2421, 2422, 2423, 2502.
Industrial air pollutants	2411.
Industrial effluents	2211, 2221, 2311, 2321, 2331, 2332, 2421.
Industrial sludge	2412.
Industrial waste discharges	2101.
Industrial waste effluents	2422.
Inorganics	2212, 2241, 2502.
Insecticides	2101, 2211, 2221, 2311, 2312, 2313. 2321, 2501.
Leachates	2101.
Metals	2213, 2215, 2224, 2231, 2324.
Multi chemicals	2214.
Monochlorinated organics	2102.
NH_3	2101.
NO_2	2101.
NO_3	2101.
NO_x	2411.
Nutrients	2101, 2102.

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INDEX XV: SAMPLE DISTRIBUTION OF THE ECOLOGICAL TEST SYSTEMS (continued)

SAMPLES	TEST SYSTEM NUMBER
Oils	2211, 2221, 2311, 2321, 2331, 2332.
Organics	2212, 2215, 2224, 2231, 2241, 2331, 2332, 2502.
Ortho-P	2101.
Other	2101.
Oxygen	2215.
Ozone	2411.
PCB's	2102, 2211, 2221, 2311, 2312, 2321, 2501.
Pentachlorophenol	2312.
Pesticides	2101, 2213, 2222, 2323, 2324, 2331, 2332, 2333, 2421, 2422, 2423.
Radiolabeled material	2501.
Receiving waters	2101, 2102.
SO_2	2411.
Tox-P	2101.
Toxic organics	2331, 2332.
Transportation related	2411, 2421.
Waste oil	2213.